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THE CONTROL OF TELIOSPORE AND UREDINIOSPORE FORMATION BY EXPERIMENTAL METHODS[1]

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INTRODUCTION

The significance to be attached to the formation of teliospores[2] following production of urediniospores in the rust-fungi has been problematical ever since this very important group of parasitic fungi has been investigated by botanists. The assumption that it is an innate characteristic which must find its expression during the course which the fungus takes in its development in the host has long been shown to be insecure, not only by common observation but by studies on the subject such as those attempted by Iwanoff (18), Morgenthaler (28), Gassner (14), Smith (40), and others.

These investigators have attempted to show by experimentation and observation that the telial generation of the rusts stands as an expression of the ability of the fungus-parasite to react to certain environmental conditions unfavorable for its continued vegetative development and to produce a fruiting structure which will tide the fungus over such adverse conditions.

Among the various causes that have been suggested as stimulating the production of such a resistant stage are direct climatic influence, temperature, moisture, resistance of the host, inner organization of the fungus, indirect climatic influences acting through the host, and so forth. However, in practically all of the experiments and observations that have been made in the past, there has been no serious effort to get at the bottom of the problem and attempt, by correlation of the results which the various factors produce, to deduce some fundamental principle which might underlie and govern the appearance or non-appearance of the teliospore generation in the rusts.

It has been the aim of this work to determine such an underlying principle and, in this paper, to analyze the results obtained and their relation to such a principle.

[1] Papers from the Department of Botany of the University of Michigan, No. 271.

[2] Since the terminology of the spore forms as suggested by Arthur (4) is gradually replacing the old, the former will be used throughout the paper.

The work was done in the Cryptogamic Laboratory of the University of Michigan under the direction of Professor C. H. Kauffman, to whom the writer is greatly indebted for generous advice and helpful criticism.

EXPERIMENTAL RESULTS

In the following work ten species of rusts were used: *Uromyces appendiculatus* Fr., on *Phaseolus vulgaris* L.; *Puccinia taraxaci* Plowr., on *Taraxacum officinale* Weber.; *Puccinia suaveolens* Rostr., on *Cirsium arvense* (L.) Scop.; *Puccinia sorghi* Schw., on *Zea mays* L.; *Puccinia asparagi* DC., on *Asparagus officinalis* L.; *Puccinia triticina* Eriksson, on *Triticum vulgare* Vill.; *Uromyces trifolii* Lev., on *Trifolium hybridum* L.; *Puccinia antirrhini* Diet. and Holw., on *Antirrhinum majus* L.; *Uromyces polygoni* Fuckel, on *Polygonum aviculare* L.; and *Puccinia orbicula* Pk. and Clint., on *Prenanthes alba* L.

Experiments were conducted with these rusts both on the hosts grown in pots in a greenhouse and on leaves or portions of leaves of the hosts grown on solutions in petri dishes in the laboratory.

Technique

In the Greenhouse

In all cases when the healthy host plants were brought in from the field and infection was obtained, checks were maintained and remained healthy. The various hosts were cultivated in pots in the greenhouse and infection obtained in a manner similar to that suggested by Melhus (26) and Fromme (12), that is, spore suspensions were made in distilled water and sprayed on by means of a No. 16 De Vilbiss atomizer, after which the plants were placed in a moist chamber for 48 hours at a temperature varying from 17° to 22° C., depending on the rust. Variations of this method were used at times, such as first spraying the plants with distilled water and then applying the inoculum with a scalpel or camel's-hair brush as advocated by Carleton (7). The latter method insures a heavier infection, but if one desires a uniform infection for comparative studies the former method is the more satisfactory.

In working with plants such as alsike, wheat, or corn, the waxy coating of the leaves prevents the spore suspension from adhering. In such cases it is necessary to draw the leaf carefully between the moistened thumb and fore-finger before applying the suspension (Melchers, 25).

In the greenhouse, various incubation chambers have been employed. Under the ordinary conditions met with during the greater part of the year, a Wardian case and bell jars have been found to be the most satisfactory. In the summer, however, it was found to be almost impossible to infect the

plants successfully by these means alone, due to the prevailing high temperatures. The "iceless refrigerator," as suggested by Hunt (17), solved the difficulty. In the refrigerator, with modifications, infection was obtained with as much ease in the hottest part of the summer as at any other time of the year. It has been found by the writer that, on a day when the temperature registered 35° C. in the greenhouse, by means of the "iceless refrigerator," it was possible to keep the plants at a temperature not exceeding 26° C. This was accomplished by opening the ventilators of the greenhouse in such a way that a breeze was constantly blowing over the surface of the refrigerator, thus increasing the evaporation and lowering the temperature. It was found, by constructing the refrigerator of sufficient size to enclose the Wardian case, that the humidity was kept higher and the plants became more heavily infected than when they were left uncovered in the refrigerator. During the spring and fall of the year when the temperature was not sufficiently high to warrant the use of the refrigerator, a metal pan was used as a cover for the Wardian case. This was kept filled with water and lowered the temperature of the case sufficiently for infection.

In applying the inoculum to the leaves of the various hosts, a difference was found in the amount of infection produced, depending upon which surface received the spores. As the stomata are, so far as we know (Ward, 46) (Evans, 11), the only means of entrance for the germ tubes of the urediniospores, the amount of infection resulting from the inoculation will vary directly with the number of stomata.

In working with infection on hosts such as corn and wheat, in which the leaves assume a more or less vertical position, the writer has been unable to find a correlation between the amount of infection and the surface receiving the inoculum, although Melchers (25) reports one-half as many stomata on the lower surface of wheat leaves as upon the upper. However, on hosts such as bean, alsike, snapdragon, etc., it was found that more severe infection was produced by inoculation on the lower surface. The snapdragon shows the greatest degree of variation, especially when leaves were inoculated in petri dishes. In the first part of the work the snapdragon leaves were always inoculated with the upper surface uppermost. The result was a very slight infection. Finally, leaves were placed in the dishes in a reversed position and the spores applied to the lower surface. Two days later the position of the leaves was changed, so as to increase photosynthesis, and just before the time for the uredinia to break through the epidermis they were again placed in the position which they occupied at the time of inoculation. Heavy infection resulted from such procedure. In all subsequent inoculations of snapdragon leaves in petri dishes, therefore, the spores were applied to the lower surface. The ideal arrangement for producing infection in petri dishes would be to place the leaves with their lower surfaces

up and have the light enter from below. This was tried by means of electric lights with noticeably increased infection. But, owing to the difficulties attending such an arrangement, it was not employed in any of the recorded experiments.

In working with plants in the greenhouse during the period of short days, the light factor became increasingly more difficult to control. With plants such as corn, beans, snapdragon, and wheat, no special difficulty was encountered. With the other hosts, however, such as alsike, Canada thistle, *Taraxacum*, and *Polygonum*, during the short days of December and January, it was almost impossible to maintain the rusts. It was only through the added influence of electric lamps, suspended a few feet above the pots, that the writer was able to keep the last-named rusts in a condition suitable for experimentation.

In addition to the difficulties that were encountered with respect to light, there arose other factors that were detrimental to the best development of the rusts. Powdery mildew made its appearance from time to time on the dandelion, alsike, wheat, bean, and *Polygonum*, and was only with difficulty controlled. Once a plant became infected with rust it was almost impossible to eliminate mildew without also killing the rust. However, by inoculating the plants with the rust and then allowing several days to elapse to insure entrance of the germ tubes, the leaves could be safely sprayed with a 1–1000 aqueous solution of sulfuric acid. The plants were later rinsed with water, and by the time the uredinia broke through the epidermis the leaf was practically free from mildew. Fromme (12) reports using this method with sulfur dust 24 days after inoculation.

In addition, insect pests added other difficulties. With the advent of the various leaf hoppers, white flies, thrips, and red spiders, the rusts at times encountered almost overwhelming competition. As it is an established fact that a vigorous host is necessary for a successful rust infection, it is essential in working with the rusts to have a good supply of healthy vigorous host plants. To obtain them, however, is not an easy matter in the average greenhouse.

In Petri Dishes

In inoculating leaves in petri dishes, the same methods were used as in the pot inoculations. In preparing leaves for inoculation, care was taken to reduce to a minimum contamination from extraneous fungi.

The leaves were first removed from the host plant and immediately placed in a dish of water. They were then washed for several minutes in running water, rinsed in several changes of distilled water, and finally placed in a dish of sterile distilled water until ready for use.

The petri dishes were sterilized in the hot-air oven in the usual manner and the leaves placed therein. In the early experiments the solutions were

always placed in the dishes at the same time as the leaves. Later experiments proved, however, that better infection resulted if the spores were sprayed on the leaves and just enough water added to the dishes to keep the air saturated for 48 hours; at the end of this time the solutions were added and the petioles of the leaves cut back in order to facilitate intake of the nutrient.

Various methods of placing the leaves in the dishes were tried out. The first method used was to place glass rods in the bottom of the dishes and allow the leaves to rest upon them. The solution was then added in such amounts that the leaves did not come in contact with the solution except for the immersion of the petiole. Another method tried was to cut circular discs of screen wire with the edges bent down in order to raise them to the proper height for the dishes and then dip them in melted paraffine to prevent rusting. The leaves were laid upon these wire discs with the petioles extending down through one of the openings into the solution. With these methods, leathery leaves became infected, but tender leaves usually died before infection took place.

Clinton and McCormick (8) used a method somewhat similar to this: rubber bands were stretched across the dish and the leaves placed upon them. These investigators encountered the same difficulties as the writer in attempting to infect delicate leaves.

The method finally used was to float the leaves freely upon the solution, either sugar or distilled water. No leaf has yet been tried which could not be kept alive and in good condition for five or six weeks with this method. When floating leaves on sugar solutions, from which the leaf is to absorb nourishment, the more cut surfaces exposed to the solution the greater the amount and the more rapid will be the absorption. This can be easily demonstrated by clearing the leaves and applying the iodine test for starch, for it is this form of carbohydrate into which the sugar is transformed for storage in most leaves. This has been shown very beautifully by Saposchnikoff (35), Acton (1), Tollenaar (44), and others. It appears that the starch is deposited centrifugally from the vascular bundles outward, the more distant cells showing the starch last. When the cut surfaces are exposed the sugar apparently travels from cell to cell by diffusion but more slowly than through the bundles. Various nutrient solutions were used upon which to float the leaves, but it was found that a five to seven per cent sucrose solution gave the best results. Ordinary commercial cane sugar was used in all the experiments, and no attempt was made to secure a chemically pure product. It was found in the course of the work that not only did the sucrose solution prove to be the form of nutrient that was best utilized by the various leaves, but it remained uncontaminated for a much longer period of time. Especially was this true of the unsterilized solution.

At first, care was taken to sterilize the solution of sucrose before it was poured into the dishes, but experience soon showed that the unsterilized solution remained free from contaminating fungi for a longer time than that which had been sterilized. This is due, possibly, to the hydrolysis of the sucrose during the sterilization process, the resulting monosaccharides offering more ready food to the contaminating fungi. Mudge (29), however, claims that practically no sucrose is inverted in an aqueous solution during sterilization. He sterilized solutions for as long as one hour under 15 pounds pressure and found practically no inversion. If this be true, the writer is at a loss to explain the uniform results obtained, unless they were due to the slight impurities in the commercial cane sugar used.

The ability of leaves and other portions of green plants to take up carbohydrates from solutions upon which they are floated has been observed by various investigators, among whom are Boehm (6), Meyer (27), Acton (1), Saposchnikoff (35, 36), Parkin (32), Atkins (5), Mains (21), and Tolenaar (44). Almost without exception they have found sucrose to be the most readily utilized of any of the sugars. They found that the optimum concentration range is from 4 to 20 per cent, depending on the conditions and type of leaf.

Among the nutrients tried during the present work were glucose, fructose, starch, maltose, raffinose, asparagin, malt extract, peptone, and cane sugar. In addition, Shive's (39) three-salt solution was added to various solutions, but the results showed that for all purposes of rust infection a simple solution of commercial cane sugar was the most satisfactory.

When leaves are kept in sugar solution, light is not necessary during storage, as the leaves absorb carbohydrates directly from the solution. Experiments, however, have shown that contamination by fungi is materially lowered when leaves in sugar solution are placed in diffused light instead of total darkness. At first they were kept under a black screen in the greenhouse, but during later experimental work they were kept on the same table as the leaves in the distilled water. In this way the leaves received the benefit of the carbohydrate solution plus any photosynthesis that may have been carried on.

The dishes containing the leaves upon distilled water had to be placed in a lighted place, as the rust-fungus obtains its nourishment only from the food which is stored in the leaf prior to inoculation or from the photosynthetic products formed in the leaf while in the dish. It was found, however, that if they were placed in direct sunlight the leaves were rapidly scalded. The plan finally used was to place them on a table in the north window where they received sufficient light to insure infection.

During the winter months when the days were short and the light in-

tensity low, electric lamps were suspended above the dishes. With the aid of these, infection was maintained satisfactorily.

The tables upon which the dishes were kept were washed thoroughly every other day with a two per cent solution of "Neko," a carbolic acid preparation (Parke Davis and Co.), to keep contamination at a minimum. The floor of the room was also washed with "Neko" occasionally. It was necessary to change the solutions in the dishes several times a week. The leaves were removed from the dishes, carefully washed in sterile distilled water, the dishes rinsed, and the leaves replaced in new solutions. If the petiole or the cut edges of the leaves appeared to be attacked by bacteria or molds, they were trimmed back so as to allow the continued entrance of the solution. The mortality rate with dish cultures is necessarily high because the leaf tissues are often bruised in handling. However, with careful observance of the rules of culture technique, a large percentage of the inoculated plants became infected. In practically all cases infection was more easily obtained on the leaves or portions of leaves in the petri dishes than on the natural host in the greenhouse.

Greenhouse Experiments with Potted Plants

The experiments given below have been grouped as far as possible according to the reactions of both host and parasite to certain environmental factors, such as light, temperature, moisture, and the resistance of the host. The influence of the food and chemical factors comes essentially from the host. As the temperature and light experiments were conducted at the same time, they will be grouped together.

Temperature and Light

The response of the host and parasite to these two environmental factors, both singly and combined, were investigated in nine rusts. They will be taken up in the order of experimentation.

Uromyces appendiculatus Fr., on Phaseolus vulgaris L. (variety: Tennessee Green Pod). In Experiment 1, twenty pots of bean were inoculated by spraying with a spore suspension and then placed in the moist chamber for 48 hours at a temperature of about 20° C. At the end of this time they were placed in the greenhouse and allowed to remain until urediniospores were formed. On the eighth day after inoculation, just as the uredinial pustules were breaking through the epidermis of the leaves, five pots were removed to a dark room at 19° C. and allowed to remain for two days. Five pots were also placed in a cold dark room at 7° C. and removed at the end of two days. The remaining ten pots were left in the greenhouse under ordinary conditions at 19–25° C. The results are shown in table 1.

TABLE 1.—*The effects of temperature and varying amounts of light on time of teliospore formation in Uromyces appendiculatus on Tennessee green pod beans*

Experiment 1

No. plants	Incubation period in days	Treatment after 8-day incubation period[a]	Formation of teliospores (no. days after inoculation)
5	8	In dark room, 19° C. for 2 days	17
4	8	In dark room, 7° C. for 2 days	15
9	8	Checks, in greenhouse at 20° C.	28

Experiment 2

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of teliospores (no. days after inoculation)
5	11	In dark room, 19° C. for 4 days	12
4	12	In dark room, 7° C. for 2 days	13
4	8	Checks, in greenhouse at 20° C.	26

Experiment 3

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of teliospores (no. days after inoculation)
3	11	In light, 7° C. for 2 days	13
3	11	In dark room, 7° C. for 1 day	13
4	12	In dark room, 19° C. for 4 days	13
4	8	Checks, in greenhouse at 20° C.	24

Experiment 4

No. plants	Incubation period in days	Treatment after 9-day incubation period[a]	Formation of teliospores (no. days after inoculation)
5	9	In light, 7° C. for 2 days	19
4	9	In dark room, 7° C. for 2 days	17
4	9	In dark room, 19° C. for 2 days	24
5	9	Checks, in greenhouse at 20° C.	28

[a] Urediniospores had formed at this time.

In explanation it may be said that under ordinary conditions of infection of bean leaves, secondary and tertiary circles of uredinia form about the primary sorus before the telial stage arises. When teliospores are formed, they usually appear in the two later-formed circles of pustules, seldom in the primary. In the above experiment, on those plants which were placed in the cold dark room, there was about 100 per cent teliospore production in the primary pustules at the end of the fifteenth day; on those which had been placed in the dark room at 19° C., there was 8 per cent teliospore production in the primary pustules; and in the case of the checks the teliospores were interspersed with the urediniospores in the secondary and tertiary pustules, with none in the primary. In the two former cases practically no secondary or tertiary pustules had formed.

These results show that both darkness and low temperature tend to suppress the uredinial stage and to favor the early production of teliospores.

In Experiment 2, the plants were removed to the dark rooms four days after inoculation to note the effect on the incubation time and also on time of teliospore formation. The results of this experiment are summarized in table 1, Experiment 2.

From the results of Experiment 2 it is seen that the incubation time is lengthened by approximately the time that the plants are kept in darkness and at low temperature.

That metabolism is not completely inhibited at 7° C. is shown by Experiment 3, in which the plants were placed in darkness at 19° C., in darkness at 7° C., and in a room at 7° C. which received light approximately 15 hours a day. In all these cases the plants were placed under the several conditions four days after inoculation. Results of Experiment 3 are summarized in table 1.

On all the plants with the exception of the checks, teliospores were found interspersed with urediniospores at the end of the incubation period.

It is apparent that light even at as low a temperature as 7° C. exerts a favorable, indirect effect upon the fungus, for the incubation period in this experiment was the same as that of the rust which was in the dark at 7° C. for only one-half the time.

In Experiment 4 the same conditions of temperature and light were used as in Experiment 3, except that the plants were placed under the changed environmental conditions at the time of the first appearance of uredinia, *i.e.*, nine days after inoculation. The results of this experiment show a relative delay in the formation of the spore forms. The incubation period was longer than usual, and the telial stage appeared later. However, the comparative results remained the same.

In the experiments thus far conducted, inoculations on the bean were always made upon the first two leaves that appeared, namely, the primordial leaves. As practically all the photosynthesis that takes place at this young stage of the plant is carried on in the primordial leaves, there is a constant withdrawal of a portion of such elaborated food for the development of the later foliage, the trifoliate leaves. By clipping back the later developing shoots it should be possible to retain a large part of this food in the primordial leaves. Thus if teliospore formation is an indication of a famished condition in the host plant, those plants that were clipped should show a comparatively longer period of urediniospore production than those that were allowed to maintain their normal growth. Accordingly, experiments were conducted to determine if this hypothesis were true.

In the next experiment, therefore, one-half of the inoculated plants were clipped and the other half allowed to retain the normal habit of growth. All plants were placed under the same environmental conditions. On those plants which had been infected and clipped, teliospores were produced from 32 to 34 days after inoculation, while on the unclipped plants the telial stage was produced from 26 to 28 days after inoculation.

The primordial leaves on the unclipped plants presented a decidedly different appearance than did those on the clipped plants. They were of a paler color and less leathery in texture, and at the time of teliospore formation showed the effects of the rust to a much greater degree than the others (Fig. 1). The sori upon the leaves of the unclipped plants were smaller, there were fewer secondary sori, and in general the leaves seemed to contain less food than those upon the plants which were clipped. The fact that the telial stage appeared on the unclipped plants from six to ten days earlier than on the clipped plants seems significant enough.

The comparative effects of temperature and light on the clipped and unclipped plants were determined by placing the plants in the several environments at the time of uredinial formation. The results are given in table 2.

TABLE 2.—*The effect of temperature and light on time of teliospore formation in Uromyces appendiculatus on "clipped" and "unclipped" plants of Tennessee Green Pod beans*

Plant no. and condition	Treatment after 7 to 8-day incubation period	Formation of telial stage	
		No. days after inoculation	Remarks
B 87–90 clipped[a]	In dark room, 7° C., for 2 days	14	In primary and secondary pustules.
B 91–93 unclipped	In dark room, 7° C., for 2 days	14–15	In primary pustules. No secondary ones found.
B 94–96 clipped	In dark room, 19° C., for 2 days	15–17	In secondary pustules.
B 97–100 unclipped	In dark room, 19° C., for 2 days	15–17	In primary pustules.
B 101–110 clipped	Checks, in greenhouse at 20° C.	30	In secondary pustules.
B 111–115 unclipped	Checks, in greenhouse at 20° C.	26	In secondary pustules.

[a] Plants with growing point removed.

These results show that, when the plants are placed in darkness at either room temperature or reduced temperature, the formation of telia is not hastened appreciably by removal of the growing point. It is only when the plant is allowed to remain under normal conditions of light and temperature and when metabolism is going on at a normal rate, with a constant increase in size of the trifoliate leaves, that the drain on the food from the primordial leaves is made apparent by the earlier appearance of the teliospore generation.

Fromme and Wingard (13) assert that in resistant varieties of beans the incubation time may be lengthened and teliospores make their appearance without being preceded by the uredinial generation as is normally the case in susceptible varieties. These results were verified by the writer in experiments conducted under normal conditions and under conditions as outlined above. The Scarlet Wax bean was used as a resistant variety.

Plants of Scarlet Wax and Tennessee Green Pod of the same age were inoculated at the same time. Nine days after inoculation the uredinia broke through the epidermis of the susceptible variety, and three days later they appeared on the resistant one. Primary and secondary sori formed on both varieties but on the susceptible one the sori were larger, more numerous, and continued to produce urediniospores for a longer time than sori on the resistant one. Teliospores appeared on the Scarlet Wax 14 days after inoculation, but it was not until the twenty-third day that they

formed on the Tennessee Green Pod. In the former case they were produced in the primary and secondary sori, while in the latter case they were formed in the secondary only. On some of the plants of the Scarlet Wax variety, teliospores were found interspersed with urediniospores in the very first pustules. In order to note the effect of unfavorable conditions on such

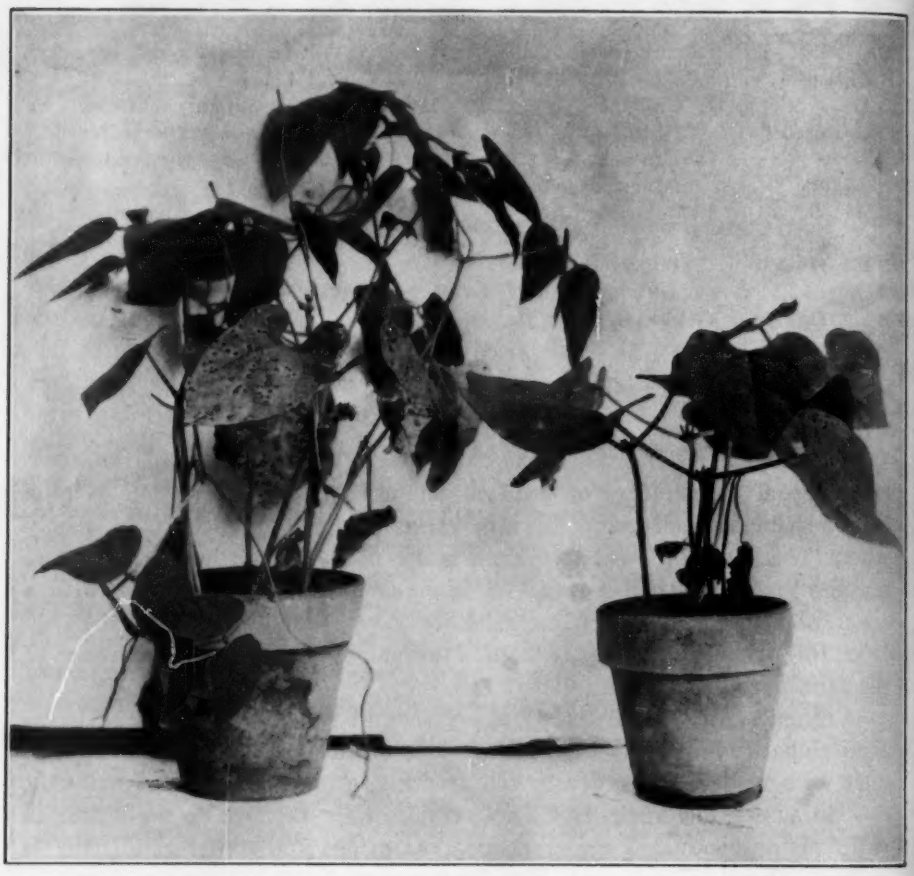

FIG. 1. Rusted bean plants, showing condition of the primordial leaves on plants with and without the growing points removed.

a host during incubation, several plants of Scarlet Wax were inoculated and four days later were placed under different conditions of light and temperatures. Table 3 gives the results of this experiment.

From the results it is evident that, when resistant varieties of beans are infected with the rust, there is a tendency for the uredinial generation to be shortened or even suppressed. When plants are placed under unfavor-

TABLE 3.—*The effect of temperature and light on time of teliospore formation in Uromyces appendiculatus on Scarlet Wax beans*

No. plants	Incubation period in days	Treatment 4 days after inoculation	Formation of teliospores	
			No. days after inoculation	Remarks
7	11	In dark room, 7° C., for 1 day	11	In primary pustules.
4	14	In dark room, 19° C., for 4 days	14	In primary pustules.
7	15	Checks, in greenhouse at 20° C.	15	In primary and secondary pustules.

able conditions during part of the incubation period, the uredinial generation may be eliminated entirely.

Puccinia suaveolens Rostr., on Cirsium arvense (L.) Scop. Cirsium rust, in nature, forms an abundance of teliospores throughout the growing season. Teliospores may be found on badly infected plants at all times, even in the primary systemic infections early in the summer.

Rostrup (34) and Olive (30) showed that *Puccinia suaveolens* was systemic and that the mycelium hibernates in the rhizome. Plants in the field infected with the primary stage of the rust never blossom, and they can be readily detected by their pale, spindly appearance. Adjacent plants are infected by the primary urediniospores which give rise to local infection, much less damaging in its effect.

For experimental material of this rust, plants were brought in from the field and planted in pots in the greenhouse. After becoming adapted to their new environment they were inoculated by spraying with a spore suspension, the spores of which were obtained from infected plants collected in the field. The incubation period of this rust varies from six to ten days, the optimum temperature for germination being about 18°–22° C.

The uredinia appear almost always on the upper surface of the leaf, although the primary uredinia usually appear on the lower. Later the sori may extend through to both surfaces. Under optimum conditions of temperature and moisture the rust may not produce teliospores for more than a month after infection. In most cases, however, the uredinia are found to contain teliospores as early as two weeks after infection. The teliospores appear on the lower leaves first and then extend progressively up the plant. This rust will not propagate itself in the greenhouse but must be re-inoculated about every 30 days in order to keep it in the uredinial stage. Under conditions of high temperature and low humidity the regions surrounding the uredinia will become dry and the rust will die.

The first experiment with this rust was conducted in order to note the effects of temperature and light on the type of spore that would be produced.

Twenty potted plants were inoculated and placed under various conditions of light and temperature similar to those used in the experiments with the bean. The results are shown in table 4.

TABLE 4.—*The effect of temperature and light on the time of teliospore formation in Puccinia suaveolens on Cirsium arvense*

No. plants	Incubation period in days	Treatment after 7-day incubation period	Formation of teliospores (no. days after inoculation)
5	7	In dark room, 19° C., 5 days	28
6	7	In dark room, 7° C., 5 days	24
10	7	Checks, in greenhouse at 20° C.	32

These results show that telial formation can be hastened by subjecting the infected plant to conditions unfavorable for metabolism in the host. When the plants were removed from the dark rooms, the areas surrounding the pustules were lighter than the remainder of the leaves and on some of the plants these areas were completely dried up.

In the next experiment an attempt was made to induce the formation of teliospores by removing the plants from pots, immersing their roots in distilled water, and placing them in the dark at a temperature of 19° C. Plants C20–25 and C25–30 were removed from the pots of earth at the time of uredinial formation and placed in flasks of distilled water, the roots being carefully washed of all particles of soil. Here they remained for two days in the sunlight, after which they were placed in the dark room for two days. At the end of this time plants C20–25 were placed in flasks of Shive's nutrient solution and returned to the sunlight, while plants C25–30 were left in distilled water and returned to the sunlight. Plants C30–35 and C35–40 were left in pots during the entire experiment (Table 5).

On plants C20–25, which were transferred at the time of uredinial formation to distilled water for two days, and then placed in the dark room for two days and finally transferred to Shive's solution for the remainder of the time, teliospores appeared on the sixteenth day after inoculation.

On plants C25–30, which were placed in distilled water at the same time as C20–25, allowed to remain for two days, and transferred to the dark

TABLE 5.—*The effect of light and nutrient solution on teliospore formation in Puccinia suaveolens on Cirsium arvense*

Plant no.	Treatment after 6-day incubation period	Formation of teliospores	
		No. of days after inoculation	Remarks
C20–25	Removed from pots and placed in distilled H_2O in light for 2 days, then in darkness for 2 days; transferred to Shive's solution and placed in light	16	Teliospores numerous.
C25–30	Do ; left in distilled H_2O, not Shive's solution	—	Pustules dried up. No teliospores.
C30–35	Potted plants in dark for 2 days, then returned to light	25	Teliospores numerous.
C35–40	Checks, in greenhouse	32	Teliospores mostly on lower leaves.

room for two days, there was no telial formation whatever. The portions of the leaves around the rust pustules dried up and a few days after removal from the dark room the rust had apparently died. The remaining plants, C30–35 and C35–40, were accorded the same treatment as plants in the earlier experiment, not being transferred from pots, and the results were practically the same.

Plants C20–25 continued to produce new leaves in the Shive's solution for 60 days after the transfer, the rusted leaves slowly drying up and dropping off, leaving the plants free from rust.

Results of other experiments similar to these were the same; in all cases the placing of infected plants in the dark either at low or room temperature was sufficient to inhibit further formation of urediniospores. In some cases, if the plants were not allowed to remain under such conditions for too long a period, uredinial production was resumed in the same pustules after teliospores had been produced. Especially noticeable was this in *Uromyces polygoni*, *Puccinia antirrhini*, and *Puccinia taraxaci*.

During winter days when the light intensity was low and the hours of daylight few, it was very difficult to maintain a stock supply of urediniospores of *Puccinia suaveolens*. When plants were infected, in many cases the first pustules that broke through contained 50 per cent of teliospores. In no case was it possible to maintain the uredinial stage for more than two weeks after the first infection. This difficulty was partially overcome by installing seven 100-watt electric lamps in the experimental room of the greenhouse. These were suspended about three feet above the pots and were supplied with reflectors to concentrate the rays of light. With the

aid of this auxiliary illumination, which was turned on from about four o'clock in the afternoon until eleven at night, it was possible to secure infection and prolong the uredinial stage to three weeks or more.

Uromyces trifolii Lev. on Trifolium hybridum L. Plants of alsike were grown from seed and inoculations made in the usual manner. The incubation time of this rust varies from 7 to 10 days, the optimum temperature for spore germination being about 18–20° C. The pustules are first evident as brownish areas on the under side of the leaves, the uredinia first breaking through on that surface, then later extending through to both surfaces. Under ordinary conditions in the greenhouse, teliospores are not formed in appreciable quantities, the leaves usually drying up before such a stage is reached. Occasionally they are formed on the petioles of the older infected leaves, and in very rare cases upon the blades, interspersed with urediniospores. This condition of course will vary, depending upon the amount of infection and environmental conditions.

The first experiment with this rust was to determine the effects of light and temperature upon the type of spore that would be produced.

Young plants, varying from four to five inches in height, were inoculated. At the time of appearance of the uredinia, some were placed in the dark at a temperature of 19° C., and others in the dark at 7° C. The results are shown in table 6.

TABLE 6.—*The effect of light and temperature on time of teliospore formation in Uromyces trifolii on Trifolium hybridum L.*

No. plants	Treatment after 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 7° C., for 4 days.	—	No teliospores formed. Leaves dried up.
4	In dark room, 19° C., for 4 days.	32	Teliospores on petioles only. Few in number.
6	Checks, in greenhouse at 20° C.	46	A few scattered teliospores on petioles.

It is evident that the treatment accorded the plants placed in the cold dark room was too severe to allow the production of any additional spores after the plants were removed. The leaves had completely withered and the plants had not grown after being placed in the dark. The host was too rapidly weakened to support the formation of teliospores. Even in the case of those plants placed in the dark at room temperature, the leaf blades had withered and the few teliospores that had formed were found upon the peti-

oles of the leaves. It was evident from these experiments that some method less drastic must be devised which would extend over a longer period of time in order to avoid killing the host too soon.

In the next experiment the plants were placed under the changed environmental conditions for shorter periods of time (Table 7).

TABLE 7.—*The effect of varying periods of light and temperature on time of teliospore formation in Uromyces trifolii on Trifolium hybridum*

No. plants	Treatment after 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 7° C., for 1 day; in dark room, 19° C., for 1 day; in greenhouse at 20° C. for 3 days; in dark room 7° C., for 1 day.	36	Some telia on leaf blades but mostly on petioles.
2	In dark room, 7° C., for 2 days.	35	Teliospores on petioles.
3	In dark room, 7° C., for 3 days.	29	Few teliospores formed. Leaves badly injured.
2	In dark room, 7° C., for 4 days.	28	Very few teliospores, on petioles. Infected leaf blades dead.
2	In dark room, 7° C., for 6 days.	—	Leaves killed. No teliospores formed.
8	Checks, in greenhouse at 20° C.	45	A few teliospores on petioles. Leaf blades dead.

In the first set of plants the gradual decrease in metabolism of the host due to the different environments was sufficient to stimulate the formation of the telial stage. That the host was not seriously injured by the treatment was evidenced by the fact that telia were formed on the blades of some of the leaves. In a similar manner the telial stage was produced on the plants which had been in the cold dark room for a period of two days. In this case, however, the blades showed the effects of the darkness and low temperature and had completely withered. The same effects were apparent in the plants which were placed therein for longer periods of time. It is evident that on this host it is difficult to reach the exact condition wherein the uredinial stage will be inhibited and the telial stage produced without killing both the host and the parasite.

As it has been shown by Acton (1), Boehm (6), and others that roots, stems, and leaves will take up carbon compounds from solution, whole plants of alsike were placed in solutions of sucrose and submitted to varying conditions of light in an attempt to lower the metabolism of the host to a point that would inhibit uredinial production and at the same time not cause the death of the host.

Sixteen plants were inoculated. At the time of uredinial formation, 12 of them were removed from the pots, the roots thoroughly rinsed and cleared of soil particles, and placed in 100 cc. flasks of seven per cent sucrose solution. The remaining four plants were allowed to remain in the pots as checks. Eight of the plants in the sucrose solution were placed in the dark room at a temperature of 19° C. and the remaining four left in the greenhouse with the checks.

The solutions were changed every day and the roots of the plants rinsed in a one per cent solution of Zonite (a commercial preparation of NaOCl held in electrolyzed hypertonic solution), after which they were thoroughly rinsed in distilled water and replaced in the new sucrose solution. In this way contamination was kept very low, and the roots did not seem to be injured by the application of Zonite.

Two days later, four of the plants in the dark room were transferred from the sucrose solution to distilled water for four days, after which they were again placed in the sucrose solution. It was noticed at this time that the petioles of the plants in the dark room contained large numbers of uredinia, while those in the greenhouse in the light had only about one-half as many.

At the end of the tenth day after inoculation, teliospores were observed on the leaf petioles of those plants which had been transferred from the sucrose solution to distilled water and back again. Three days later they were also found on the plants which had been in the dark continuously on the sucrose solution. They were far more numerous on the latter plants, each pustule containing 50 per cent teliospores. On the former the teliospores were few and interspersed with urediniospores in only about 30 per cent of the pustules. Three days later teliospores appeared on the plants in sucrose in the greenhouse, and on the potted plants in the greenhouse on the thirty-eighth day following inoculation.

In practically all cases in this experiment teliospores were formed on the petioles and not on the leaf blades. The leaves of the plants in the dark withered soon after they were placed there, the ones on distilled water dying before the others. It appears that the plants which were on the sugar solution continuously in the dark, although they did not produce the telial stage so soon as those which were transferred to water, absorbed more of the nutrient from the solution and as a result produced more teliospores than

the other plants. The plants on water felt the hunger stimulus first but did not have the food supply to produce the abundant crop as did the others.

Puccinia taraxaci Plowr. on Taraxacum officinale Weber. Taraxacum rust is similar to *Puccinia suaveolens* in that it produces pycnia accompanied by primary uredinia instead of by aecia. Infections by primary urediniospores produce the regular secondary urediniospores followed by the telial stage. The incubation period of *P. taraxaci* varies from 8 to 12 days; the uredinia first appear on the lower surface, and usually break through the upper epidermis later. The optimum temperature for spore germination appears to be about 18–20° C.

The telial stage of this rust is seldom found in the greenhouse and, according to the experience of the writer, has never been seen there except under experimental conditions as described below. There appears to be great variability in the resistance and susceptibility of host plants to this rust, for plants have been brought into the greenhouse which have never become infected as a result of inoculation, either in pots or petri dishes. Other individuals transferred from the same vicinity in the field showed great susceptibility under both conditions. In the work with this rust, as well as with the others, care has been taken to limit the experimental work to one strain of rust, so that, to the best of the writer's knowledge and belief, discrepancies from this source have been kept out. Any difference in infection ability has been due to the host alone.

Various experiments were tried with light and temperature, similar to those already reported, but in no case under these ordinary methods was the telial stage produced. An infected plant may continue to produce urediniospores until the leaf is entirely covered with pustules. Placing the plants in darkness and under low temperature may cause a temporary lessening of uredinial production, but shortly after the plants are again placed in a favorable situation copious uredinial production is resumed.

Infected plants were placed in the dark at a temperature of 19° C. and also in the dark at 7° C. and left for periods varying from one to seven days, but in no case were teliospores produced. In extreme cases the host was killed without ever showing any signs of the resistant spore. In all, 65 infected plants were used, and not a single teliospore was produced.

Finally an attempt was made to vary the conditions in such a way as slowly to bring the metabolism of the host plant to a condition similar to that existing in nature during the late growing season when the telial stage is produced.

Taraxacum plants were inoculated. Three days after the first appearance of uredinia, plants T10–15 were placed in the cold dark room at 7° C.

for three days. They were then transferred to the greenhouse for seven days and again removed to the cold dark room for six days. Five days after they had been returned to the greenhouse, teliospores appeared in the old uredinial pustules. They were not numerous, averaging about one teliospore to every ten urediniospores. They continued to form for four days, until, under the influence of the greenhouse environment, urediniospores were again produced exclusively. The plants were then transferred to the cold dark room for three days and, three days after their removal to the greenhouse, urediniospore production ceased and teliospores were again formed in the old pustules. This continued for several days to the exclusion of the urediniospores, until finally the infected leaves died, leaving the plant rust-free.

This experiment shows that it is possible to induce telial production even in those plants which do not produce the telial generation readily under the ordinary experimental methods used for some of the other rusts. The principle, however, is the same in this case as in the others.

Puccinia antirrhini Diet & Holw., on Antirrhinum majus L. Plants of *Antirrhinum* were raised from seed in the greenhouse and inoculations made in the usual manner. The incubation period of *Puccinia antirrhini* ranges from 7 to 14 days. The optimum temperature for spore germination is about 19–20° C. A temperature of from 25 to 30° C. will gradually free the host of the parasite.

The first evidence of infection on the host plants are light areas on the under surfaces of the leaves. These gradually give way to the uredinia, which seldom extend through to the upper surface. The uredinia are large and circular, and under optimum conditions increase in a radial direction by forming concentric rings of uredinia outside of the original sorus. The uredinial and telial generations are the only ones that have been observed on *Antirrhinum*, and, until Hackey (15) and Mains (23) succeeded in germinating teliospores, they were generally thought to be functionless.

Under ordinary greenhouse conditions, teliospores are seldom produced on actively growing plants. Doran (10) succeeded in producing them on plants which had been gradually deprived of water. This probably accounts for their presence occasionally on old stems of infected plants which have lost their leaves in the greenhouse.

Doran (10) reports finding teliospores on plants outdoors after the low temperature of autumn had set in. The writer has found the telial stage both on the leaves and stems in the field in November, although telia were scarce on the leaves.

The first attempt to inhibit the continued production of urediniospores and so obtain formation of teliospores was made in the same manner as for

the earlier mentioned rusts. Plants were placed in the cold dark room immediately on the appearance of uredinia and allowed to remain at 7° C. for periods of time ranging from 2 to 6 days. On being returned to the greenhouse, the urediniospores continued to form on those leaves which had not been seriously injured by the low temperature. By varying the time left in the dark and allowing the plants partially to recuperate in the light after each exposure to low temperature, teliospores soon made their appearance. The results are given in table 8.

TABLE 8.—*The effect of light and temperature on the time of teliospore formation in Puccinia antirrhini on Antirrhinum majus*

No. plants	Treatment after 10-day incubation period	Formation of teliospores (no. days after inoculation)
4	In dark at 7° C. for 3 days; in light at 20° C. for 10 days; in dark at 7° C. for 3 days	21
5	In dark at 7° C. for 4 days	No teliospores.
5	In dark at 7° C. for 6 days	do
8	Checks, in greenhouse at 20° C.	do

The telial stage appeared only on those plants placed at low temperature in darkness at two different times, with an interval between; this permitted the host to withstand the effects of the unfavorable environment without serious injury. On the plants which were subjected to the same treatment only once, there were no teliospores. It would appear that, similar to the case of the Taraxacum rust, it required a more extended and gradual treatment to upset sufficiently the metabolism of both the host and the parasite so that the latter was stimulated to produce teliospores. The tips of the infected leaves on all of the plants were withered, and it was only back of these withered areas that teliospores were produced. They were in most cases interspersed with urediniospores, but on a few of the leaves separate telia were formed near the withered tips. It appeared as if the mycelium of the parasite were spreading from the generally infected area back into the uninfected regions of the leaf and there encountering the conditions which stimulated it to produce teliospores. The region of the leaf surrounding the telia was not withered and did not have the appearance of being seriously injured. The regions around the uredinia were severely injured, and it appeared as if these parts of the leaf had succumbed so rapidly that the fungus did not have time to produce the resistant spores.

In the next attempt to produce teliospores, eight plants were transferred, at the time of urediniospore formation, from pots of earth to flasks of distilled water, the roots only being submerged. The flasks were placed in the

dark room at a temperature of 22° C. for three days. At the end of this period the plants were repotted and returned to the greenhouse. Ten days later teliospores appeared in the same manner as before; most of them were interspersed with urediniospores, but a few were in separate telia. They did not continue to form for any length of time, and the same sori, three days later, disclosed the formation of new urediniospores on the outer edges of the old pustules. Thus it appears that, after the stimulus which had induced the formation of teliospores had been removed, the same mycelium was capable of resuming the production of the uredinial generation.

Uromyces polygoni Fuckel, on Polygonum aviculare L. Rusted plants of *Polygonum aviculare* were brought in from the field near Ann Arbor and planted in pots in the greenhouse. Uninfected plants from this general region were also placed in pots and inoculated with material obtained from the infected hosts.

The incubation period of *Uromyces polygoni* varies from 8 to 15 days, the uredinial sori first breaking through the leaves on the lower surface and later extending through to the upper. This rust is exceedingly prolific and will spread over an entire plant in a short time even in the greenhouse. The telial stage forms very readily and is produced upon old leaves of infected plants during the entire growing season.

Infected plants P1–5, on the first appearance of the uredinial stage, were placed in the dark room at 7° C. for four days, afterwards being removed to the greenhouse with the checks. Plants P5–10 were placed in the dark room at 19° C., at the same time for the same length of time.

Four days later teliospores were found on plants P1–5 and plants P5–10. In the case of the former, the production of teliospores was almost 100 per cent on all of the infected leaves. They were produced in the old uredinial pustules and were evident only after the old urediniospores had been scraped away. On plants P5–10 they were not so numerous and were only formed on those leaves which were older and more heavily infected. On the checks teliospores were not yet being produced except on some of the lower, older leaves which had been severely injured by the rust.

In four days urediniospores could be found again on all of the plants, the majority being produced around the outer margins of the old pustules. The plants were again subjected to the same conditions as before, and three days after removal to the greenhouse teliospores were being formed for the second time. They were produced on plants P1–5 until the infected leaves dropped and left the plant rust-free. On plants P5–10 teliospores were produced for several days, then urediniospores were formed again for a short time before the rust finally died. On the check plants, meanwhile,

urediniospores were produced, except on the older infected leaves, for several weeks, until gradually the rust died.

Puccinia triticina Erikss., on Triticum vulgare Vill. Plants of the Golden Wave variety of wheat were grown from seed in pots in the greenhouse and infected with material of *Puccinia triticina* received from Dr. E. B. Mains, of the Purdue Agricultural Experiment Station, Lafayette, Indiana. The incubation period varies from 6 to 12 days. The first signs of infection are light-colored areas on the leaves, through which the uredinia soon break. Leaves infected with *P. triticina* soon dry up, and unless they are reinoculated about every 15 days it is impossible to maintain the rust in the greenhouse.

In all of the experiments made with this rust in the greenhouse, the writer was not able to produce a condition of the host whereby the uredinial stage was inhibited and the telial stage stimulated, although such a condition is not to be considered impossible of attainment in greenhouse experimentation.

Rusted plants were subjected to darkness, low temperatures, dessication, etc., but in no case were teliospores produced. They were, however, induced on detached leaves in petri dishes. A description is given later.

Puccinia sorghi Schw., on Zea mays L. The effects of light and temperature upon the production of teliospores by *Puccinia sorghi* was demonstrated by several experiments similar to those conducted for rusts so far mentioned.

Mains (21) carried on exhaustive cultural experiments with this organism and gives an account of its reactions, so that further discussion of the preliminary procedure is unnecessary.

Plants were inoculated in the usual manner, and at the time of first uredinial formation were put in darkness and at low temperatures. The results are shown in table 9.

In this situation we find a condition somewhat similar to that present in the wheat. It is difficult to adjust the conditions for control of the metabolic processes in the host in such a manner that the rust will be able to respond without first killing both the host and its parasite. On only one of the four plants placed in the dark room at 19° C. were teliospores produced, and on two of the four placed in the dark room at 7° C. In both of these cases they were very scattered and were interspersed with urediniospores in the center of the uredinia. In the other plants either the infected leaves died, or the region surrounding the sorus appeared to be completely dried out, leaving the rust mycelium isolated from the food in other regions of the leaf. It is interesting to note that in infected leaves exposed to light the areas surrounding the sori retain their green color long

TABLE 9.—*The effect of light and temperature on time of teliospore formation in Puccinia sorghi on Zea mays*

No. plants	Treatment after 6 to 8-day incubation period	Formation of teliospores	
		No. days after inoculation	Remarks
5	In dark room, 19° C., for 4 days	—	Leaves dried up. No teliospores formed.
6	In dark room, 19° C., for 3 days	—	do
4	In dark room, 19° C., for 2 days	20	A few teliospores formed in with the urediniospores on one of the plants.
5	In dark room, 7° C., for 3 days	—	Leaves dried up. No teliospores formed.
4	In dark room, 7° C., for 1 day	17	Teliospores formed on two of the four plants in the old uredinial pustules.
10	Checks, in greenhouse at 20° C.	—	No teliospores formed. The older leaves gradually dried up and left the plant rust-free.

after the other portions have faded, while the reverse is true of plants placed in the dark.

This phenomenon has been observed by Ward (48), Tubeuf (45), and Mains (21) in their experiments with rusts.

Puccinia asparagi DC., on Asparagus officinale L. Seeds of asparagus were planted in pots in the greenhouse, and when the plants were about eight inches tall they were inoculated with *Puccinia asparagi* collected in the field near Ann Arbor.

The incubation period of this rust is quite variable, depending largely on the age and condition of the host. Very small plants are not easily infected. In several of the inoculations the time was from 12 to 18 days. In petri-dish culture, to be taken up later, the incubation time ranged from 12 to 20 days.

In all the experiments on the effect of temperature and light the results were negative as far as production of teliospores was concerned. Infected plants were subjected to various conditions but in all cases died befor any results could be expected.

Moisture Relations of Teliospore Production

The moisture relation of *Puccinia asparagi* can, according to Smith (40), be considered under two heads; the direct effect of atmospheric moisture on the spores or mycelium of the fungus, and the indirect effect of moisture on the parasite through its effects upon the host. The latter, of course, means soil moisture.

The writer has attempted to verify by experimental methods in the greenhouse the field observations of Smith upon the moisture relations of *P. asparagi*.

Potted plants of asparagus were inoculated with the uredinial stage of the rust and the infected plants apportioned for the experiments as follows:

Two of the plants, A1–2, were placed in the greenhouse where the humidity was relatively high and given only enough water to keep them alive. Two, A3–4, were placed under bell-jars in a well-lighted place with the pots standing in water. The remaining two, A5–6, were placed in the greenhouse by the side of A1–2 and kept well watered.

One week later, on plants A1–2 teliospores appeared in the center of the old uredinial pustules. In another week the entire infected regions of the stalks were filled with teliospores and the fungus had ceased to spread.

On plants A3–4 the rust continued to form urediniospores for two weeks, new sori appearing as concentric circles around the old. The urediniospores were heaped up in the sori with exceedingly long pedicels, and behaved as the rust did when cultivated in petri dishes on detached stalks of asparagus. This is probably due to high humidity, since Hennings (16) observed the same conditions on rusted plants in very humid environments. During the third week after infection teliospores appeared in the center of the first formed uredinia and gradually spread outward but with much less rapidity than in plants A1–2. By the end of the fourth week the pustules were exclusively telial and the infected stalks were killed.

On plants A5–6 teliospores were produced at the end of the second week in the center of the first formed uredinia. The former spread outward in a radial direction preceded by the circles of uredinia. By the end of the third week the sori were completely made up of teliospores and the stalks were dead.

We thus get different results under three sets of different environmental conditions: first, high humidity and high soil moisture; second, moderate humidity and high soil moisture; third, moderate humidity and low soil moisture.

Under the first set of conditions, prolific urediniospore production occurred for nearly three weeks before the telial stage appeared, and even then the new uredinia continued to form for almost two more weeks before teliospores were produced in all of the sori.

In the second case teliospores appeared during the second week, preceded by uredinial production until the end of the third week.

In the last situation the telial stage appeared during the first part of the second week, and before another week had passed the pustules were completely telial in nature and the spread of the fungus had ceased.

It is evident that soil moisture played an important rôle in determining the type of spore produced, for plants A1–2 and A5–6 were exposed to precisely the same humidity, but the amounts of soil moisture differed. That humidity played a part is also evident, as plants A3–4 and A5–6 had practically the same amounts of soil moisture but different humidities.

Four more potted asparagus plants were inoculated and, at the time of uredinial formation, two of them, A12–13, were placed in the south window of the laboratory room, while the other two, A14–15, remained in the greenhouse. Both sets of plants received plenty of soil moisture, practically the only difference in environment being the relative amounts of humidity.

Plants A14–15 developed in the same manner as did A1–2 in the previous experiment, the uredinial sori spreading radially, followed by the production of teliospores in the old pustules. By the end of three weeks spore production had almost ceased, teliospores occupying the whole of the sori.

Plants A12–13 formed teliospores at the end of seven days in the primary and secondary sori previously occupied by urediniospores. No uredinia formed beyond the second ring, and by the end of two weeks the spread of the rust had been checked. The plants were then taken from the laboratory and placed under bell-jars in the greenhouse with plenty of soil moisture in the pots. Six days later new uredinia were being formed exterior to the old sori, followed shortly by teliospores.

The results of the latter experiment added to those of the previous one make it certain that moisture, both in the air and soil, plays an important part in the type of spore produced by the fungus. However, can we say that either has a direct effect upon the fungus parasite? Until we know exactly what influence the shifting of environment had upon both the host plant and the parasite, we are not justified in ascribing to moisture of any kind a direct influence upon the fungus alone. Smith (40) says that when green stalks containing telial material are placed in moist chambers "there immediately breaks out at the outer edge of the infected area a circle of uredosori, with spores capable of immediate germination." This he ascribes to the direct effect of atmospheric moisture upon the rust fungus, without considering that back of the fungus is the living host with its complex of metabolic processes. Did the placing of the stalk in the moist chamber change the metabolic activities of the parasite and leave completely unchanged the cells of the living substratum upon which the fungus was growing? Certainly, from a physiological standpoint, the host in the moist

chamber was a different host than the one on which the teliospores were being produced. To the writer, it appears that, until we can separate the rust parasite from its obligate host and culture it upon an artificial medium, we cannot be justified in assuming that a change in environment of any kind will directly affect the fungus without at the same time having an equal effect upon the cells of the living host. These, in turn, through their change in metabolic activity, will exert a far greater influence upon the obligate parasite than any external factor such as moisture, light, temperature, etc.

Inasmuch as we can safely assert that the living host cells are the sole means of food supply for the rust fungus, and as the increased activity of such cells in a congenial host increases the activity of the parasite, any influence that is favorable for the host cells will be correspondingly favorable for the fungus. Thus when asparagus plants were brought from an environment of low moisture to a more favorable one of high moisture, metabolism increased—more food was made available for the rust—with the result that the uredinial generation was again produced.

In the case of bean rust the moisture relations, while not so marked as in the asparagus, nevertheless were noticeable. On rusted plants slowly deprived of water the telial stage appeared earlier than on plants which were kept well supplied.

Infected bean plants were divided into two lots, one brought into the laboratory at the time of uredinial formation and placed in an east window, the other allowed to remain in the greenhouse. Both were kept well supplied with soil moisture.

Three weeks from the time of inoculation, on the plants in the laboratory, teliospores were forming in the old primary uredinia, while none appeared on those in the greenhouse for another week. In the latter case secondary and tertiary uredinia had been formed, and it was in the tertiary pustules that teliospores appeared. While the light factor may have entered into the experiment to some extent, it was not sufficient to account for the extreme contrast in results. Repeated experiments produced the same results; even when additional light was supplied to the plants in the laboratory by means of electric lamps, the teliospores always appeared from five to seven days sooner than on the plants in the greenhouse.

In the case of snapdragon rust, Doran (10) has already shown the effect of moisture on production of teliospores. In the experiments conducted to repeat those of Doran, the teliospores always made their appearance on the stems and never on the leaves. Infected plants, with uredinial lesions on both the leaves and stems, were gradually deprived of water, and in four weeks after the treatment was begun teliospores were produced on the stems. The leaves meanwhile had dried up, only the uredinial generation

having been produced on them. Those plants which were kept well supplied with water were always killed by the rust before the telial stage was reached.

Petri-Dish Experiments

Eleven rusts, including the nine already mentioned, have been cultured on leaves or portions of leaves floated on water or aqueous solutions in petri dishes. Both urediniospores and teliospores have been produced in eight of these rusts, the type of spore depending in each case on the treatment to which the leaves of each host were subjected. In all the experiments recorded, a five or seven per cent cane sugar solution was used, the former being employed only in the first experiments with bean rust.

Uromyces appendiculatus Fr., on Phaseolus vulgaris L. Bean rust is exceedingly easy to culture in dishes and forms its telial stage very readily when placed under conditions that are unfavorable for the host. It is a mistaken notion to think that a weakening of the host predisposes it to the ravages of a rust. Quite the contrary is true. In order to insure a well developed growth of a rust it is quite essential that the host, presuming it to be a susceptible one, be in a healthy vigorous condition (Arthur, 3, and Stakman, 41). If the host is weakened or stunted, the rust either will not develop or will be weakened and produce small pustules and pale spores, and in some cases the uredinial stage will be shortened and the telial stage produced prematurely. A fairly vigorous condition of leaves can be brought about by the use of nutritive sugar solutions; hence it is possible to use the petri dish method for a study of rust reactions.

A few preliminary experiments with bean rust have already been reported upon by the writer (50), and these have shown the ease with which the spore stages can be controlled. During the first experiments it was noted that when bean leaves on distilled water were inoculated, the uredinial pustules were always smaller and paler than when the leaves had been floated on a sugar solution. On leaves on distilled water the pustules seldom increased in number as they did under favorable conditions, as when leaves were on sugar solutions. When teliospores were formed on the leaves on water they usually appeared in the primary pustules, and the rust died in from five to seven days after the first appearance of the uredinial stage. On the other hand, when the leaves were on sugar, secondary and tertiary pustules were usually formed, and when teliospores did make their appearance they were nearly always formed in the two latter sori, seldom in the primary. Quite commonly the rust became so active that the leaf was killed without ever forming teliospores. Frequently the leaf was a mass of uredinia with the spores heaped up to a height of a millimeter or more in the sori. Under such conditions telia were seldom formed.

In all of the dish experiments, the amount of infection played an important part in the appearance or non-appearance of teliospores. A light infection with few sori usually meant a long continuance of the uredinial stage if the leaf was kept on the sugar solution; changing such leaves from sugar to distilled water usually hastened somewhat the appearance of the telial stage. Frequently the reverse was also true. An infected leaf which had been on distilled water was likely to produce the telial stage when suddenly transferred to sugar solution.

Young primordial leaves were taken from healthy bean plants and placed in dishes containing a small amount of distilled water. They were sprayed with a spore suspension and set aside for 48 hours. At the end of this time a seven per cent sucrose solution was poured into one-half of the dishes in sufficient amounts to float the leaves freely. The petioles were clipped and the ends immersed to facilitate the entrance of the nutrient. At the time when uredinial pustules were just appearing on the leaves on sucrose, one-half of them were rinsed thoroughly and placed on distilled water. Likewise one-half of those on distilled water were placed on the sugar solution. The leaves were then set aside for seven days, at the end of which time they were examined.

The following scheme will be followed for designating the various treatments given the leaves in dishes:

A—Leaves which had been continuously on distilled water.
B—Leaves which had been on sucrose continuously.
AA—Leaves which had been transferred from sucrose to distilled water.
BB—Leaves which had been transferred from distilled water to sugar.

A. A fair amount of infection was obtained, but the sori were small and pale in color. The leaves were in a good state of preservation and did not seem to be damaged to any great extent as a result of the rust. In the pustules a few teliospores were interspersed with urediniospores, the latter predominating.

B. Heavy infection was secured, with an abundance of secondary uredinia. The leaves were considerably faded with the exception of the regions immediately surrounding the sori. No teliospores were found.

AA. Infection was almost as heavy as in B, but there were fewer secondary uredinia. Teliospores were abundant in the secondary pustules.

BB. The heaviest infection of all was obtained. There were not so many secondary uredinia, but the primary sori were more numerous and larger than those on the other plants.

One week later a second examination of the dishes was made, with the following results:

A. Growth was very scanty; the sori were very small; and comparatively few secondary pustules had formed. The sori, both primary and secondary, except on those leaves which had unusually heavy infections, contained teliospores almost entirely. The heavily infected leaves had no teliospores; they were in an impoverished condition, and the rust appeared to be famished to a point where it no longer had the ability to form teliospores. Leaves on which teliospores had formed and on which the infection was only moderate, were still green and did not appear to be injured by the parasite.

B. Many of the leaves were yellow and dead. The secondary and tertiary pustules contained an abundance of teliospores. As in A, in cases of very heavy infection, no teliospores were formed, and from the appearance of the leaves it seemed reasonable to suppose that the fungus developed with such intensity and speed that the cells of the host were killed and the fungus was practically isolated from the food supply and perished without the time or ability to form the resistant spore.

AA. There was practically no change in the development of the parasite. The leaves were almost entirely yellowed, and the production of teliospores in the primary and secondary sori had not increased much since the last examination. It would appear that, when water replaced the sugar solution, the fungus gradually stopped growing and producing teliospores.

BB. There was further deterioration of the leaves with not much change in the development of the fungus except that teliospores had formed in the secondary and tertiary sori. The areas immediately surrounding the sori were still green in color but did not appear to be alive. It appeared that this area had been isolated from the surrounding tissue and had dried up without losing its color.

The appearance and condition of the leaves of the bean, both in dishes and when attached to the plant, at the time of teliospore formation, suggested a test for the amount of stored food in the leaf at such a period. As the bean leaves store large amounts of transitory starch and practically no sugar, it seemed reasonable to suppose that, even though the rust existed upon some transitory products of photosynthesis or even upon food of a protein nature, the presence or absence of starch in the leaf would give very good indications of the food situation in the leaf. Accordingly, starch tests were made on leaves from the host plant and in petri dishes at different stages in the development of the rust, *i.e.*, when producing urediniospores and also when forming teliospores. The evident correlation between the amount of starch in the leaf and the type of spore produced at that time seemed to be strong proof that the appearance of teliospores on bean leaves indicated a paucity of carbohydrates in the host cells.

The results of these tests follow:

1. Leaves from plants in the greenhouse tested two days after the first appearance of the uredinial stage had large amounts of starch throughout the leaf. (Leaves for all tests were gathered at four o'clock in the afternoon, so that in these comparative tests translocation had not progressed to any great extent.)

2. In leaves from greenhouse plants tested 10 days after the appearance of the uredinial stage, the starch areas were localized around the uredinia. The areas distant from the fruiting pustules showed little or no starch.

3. Tests made of leaves 28 days after inoculation and bearing teliospores gave almost no test for starch. Even the areas surrounding the pustules gave only a faint starch reaction.

In plants which had been clipped, the reaction for starch was evident for longer periods than on those plants which had retained their trifoliate leaves. In general, the amount of starch in the leaf varied inversely with the number of teliospores.

Tests made on leaves which had been floated on sucrose solutions and distilled water in petri dishes showed the same variation:

1. Lightly infected leaves on distilled water showed a fair starch reaction at the time of uredinial formation.

2. Leaves on distilled water with heavy infections showed little or no starch at the time of uredinial formation. Such leaves usually had no teliospores.

3. Infected leaves on seven per cent sucrose solution gave an extremely strong starch reaction at the time of uredinial formation, the starch being denser along the cut edges of the leaves and along the larger veins.

4. Infected leaves on sugar at the time of telial formation had little or no starch. In cases of heavy infection where no teliospores were formed, there was practically no starch.

Attempts were made to show the effects of low temperatures upon the formation of teliospores similar to experiments made upon the plants in the greenhouse, but the results were negative.

It was found, on bringing the leaves from low to high temperatures, that rapid decomposition set in, which made it impossible to work with them. Another reason for the inability to perform temperature experiments with detached leaves is the fact that at low temperatures very little starch is formed in the leaves and the development of the rust is at a standstill. The reaction of leaves and their storage of starch at low temperatures has been investigated by Czapek (9), Lidforss (19), and Tolenaar (44), who found that starch storage took place at low temperatures to a much less degree than at high.

Uromyces trifolii Lev., on Trifolium hybridum L. Infection of alsike leaves with *Uromyces trifolii* was easily obtained either on distilled water or the 7 per cent sucrose solution. In the former case the pustules were smaller and paler in color than those formed on the leaves on sucrose. That alsike leaves take up sugar from solution and store it as starch was made evident by iodine tests similar to those made on the bean. Figure 2 shows the result of one such test.

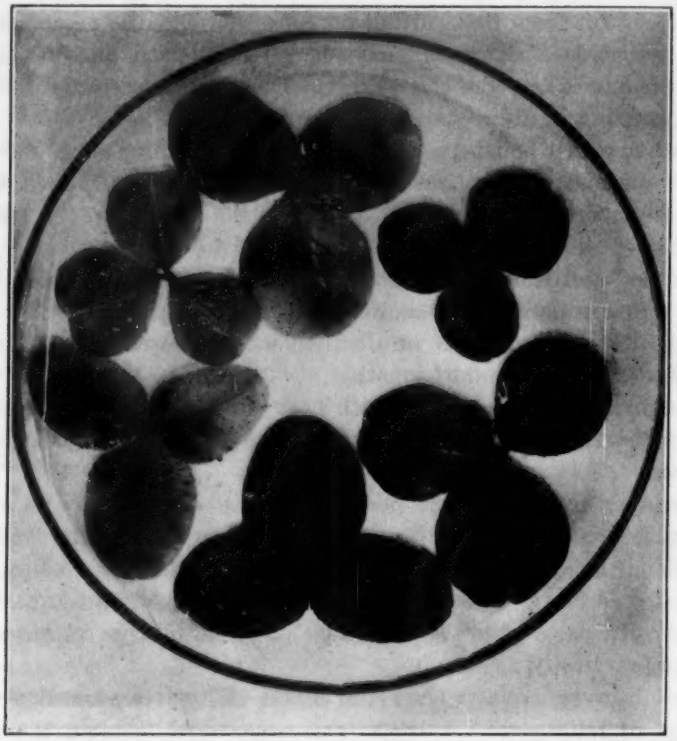

FIG. 2. Starch tests made on leaves of alsike bearing the uredinial stage of the rust The three leaves on the left were floated on distilled water while the three on the right were floated on a seven per cent sucrose solution.

Preliminary experiments were conducted with alsike leaves by placing some of the dishes containing the leaves in the dark and others in the light during incubation. It was found that with leaves floated on sugar solutions good infections could be obtained if they were kept in total darkness during the whole period of incubation. In the case of the leaves on distilled water, however, it was necessary to place them in a well-lighted position to insure good infection. In some few cases scattered pustules were formed on those

leaves which had remained in the dark during incubation, but this can be attributed to the fact that there was a sufficient amount of stored food already in the leaves to insure at least a scanty infection. Unlike the situation found in the bean leaves, teliospores were found in only two cases out of hundreds of trials with leaves on distilled water. In these exceptions, the number of sori was very few on the leaf, in one instance numbering 20, in the other, 28. Normally, by the spray method of inoculation, 2 to 500 pustules are usually formed on a single leaf; hence it appears to be a case of the fungus famishing before being able to produce the telial stage.

To induce the formation of teliospores, various methods were tried. It has been shown in the experiments with the various host-plants in the greenhouse that exhaustion of the food supply in the host, either through lack of light or moisture, or low temperatures, seemed to inhibit further production of urediniospores with the resulting formation of the telial stage. Methods were therefore used in the petri-dish cultures to control the food supply of the host. It was found that teliospores could be induced in the leaves of alsike by three methods:

1. Starvation of the host, with the later addition of a rich food supply. *2.* Sudden change of the host from a well-fed condition to a stage of starvation. *3.* Continued supply of food, with the gradual dying of the host cells and lowering of metabolism. These objectives were attained by the addition and subtraction of the nutrient solutions. The efficiency of the method is, of course, much lower in this case than in working with fungi which can be cultured directly on artificial culture media, because we have the living host, with all of its complexities of metabolism and growth, interpolated between the nutrient solution and the fungus. Nevertheless, sufficient data were secured to justify the conclusions. The attempt was also made to show the effects of low temperature on the leaf and rust metabolism as in the case of the bean leaves, but the same difficulties were encountered and that phase of the experimentation was suspended.

Table 10 shows the results of infection experiments conducted with detached leaves. The results show a relationship between the presence of carbohydrates in the leaves and successful infection.

Experiments were next conducted to show the effects of changing the nutrient solutions on the infected leaves. Alsike leaves in 32 dishes were inoculated, and at the end of 48 hours sucrose was added to 16 of them, the other 16 receiving distilled water.

Eight days after inoculation, uredinia appeared on the leaves on the sucrose solution and one day later on the leaves on distilled water. At this time seven dishes of leaves were changed from distilled water to sucrose, seven were allowed to remain on the water, six were changed from sucrose

TABLE 10.—*The effect of light and medium on development of Uromyces trifolii on detached leaves of Trifolium hybridum*

No. dishes containing detached leaves	Medium	Incubation period in days	Light condition during incubation	Infection
12	Distilled H_2O	7	Light	Sori small and pale in color.
8	do	10	Dark	Only two out of eight leaves infected.
8	7% sucrose solution	6–7	Light	Good infection; 200–500 pustules per leaf.
8	do	7	Dark	Fair infection, large pustules; 50–100 pustules per leaf.

to water, and ten remained on the sucrose. The solutions were changed every other day to prevent contamination.

Twenty days after inoculation the leaves were examined. The results were as follows:

1. The leaves which had been on distilled water continuously were all lightly infected, and on one leaf that had a lighter infection than the others there were teliospores formed in the old uredinial pustules and interspersed with the urediniospores. The other leaves were in a fair state of preservation, but the rust was not developing any further.

2. On the twenty-second day after inoculation teliospores were found in five of the seven dishes of leaves that had been transferred from water to sucrose. The teliospores were quite numerous and in some cases were formed in separate telia; the majority, however, were formed in the old uredinial pustules.

3. Examination of those leaves which had been on the sucrose throughout the experiment revealed only a very few teliospores. Uredinia were formed in very large amounts and the leaves had the appearance of being almost destroyed. As was found to be the case with most of the rusts, a continued supply of nourishment almost universally caused the fungus to produce the uredinial stage until finally the host was exhausted so suddenly that there was no time for the formation of teliospores.

4. The rust on leaves that had been transferred from sucrose to water had grown practically not at all after the sucrose was removed. For several days after the nutrient was taken away a few urediniospores had been formed, but not many. No teliospores were formed, and the leaves soon disintegrated.

As no teliospores formed when the leaves were transferred from sucrose to water at the time of uredinial formation, it was thought advisable to transfer them before the fungus could progress that far in its development and before it could appropriate too much of the stored food in the leaf for the production of urediniospores. Accordingly, leaves in 16 dishes were inoculated in the regular manner and set aside for only 48 hours. At the end of this time, the leaves in eight of the dishes were supplied with 7 per cent sucrose solution, the leaves in the other eight remaining on distilled water. Six days after inoculation, leaves from four of the eight dishes were rinsed and transferred from sugar to distilled water.

From four to six days later, uredinia appeared on practically all of the leaves in the 16 dishes. Twenty days after inoculation teliospores appeared on the leaves which had been transferred from sugar to water. The other leaves showed about the same results as those outlined in the preceding experiment. The fact that teliospores formed on leaves which had been transferred from sugar to water before uredinia had formed made it appear that the sudden removal of food at such a stage of development inhibited production of urediniospores and stimulated the immediate formation of teliospores.

Puccinia suaveolens Rostr., on Cirsium arvense (L.) Scop. Puccinia suaveolens was not at first readily cultured on leaves of *Cirsium arvense* in dishes due to their rapid discoloration and decomposition. In the early part of the work it seemed almost impossible to obtain infection. Finally it was found that by selecting the younger and more succulent leaves for inoculation a large percentage of them became infected.

Experiments were conducted to determine the ability of the Cirsium leaf to take up sugar and store it as starch in the cells, and it was found that large amounts were stored after four or five days on the 7 per cent sucrose solution.

Infections of rust on leaves floated on distilled water, if not too heavy, produced teliospores a few days after the first appearance of the uredinial stage. During winter days when the light intensity was low it was not infrequent to find that the telial stage was the first and only stage of the rust to appear on the leaves on water.

That the presence or absence of food in the leaf was a deciding factor in teliospore production is shown by the results of the following experiment. Leaves in 28 dishes were inoculated, and 48 hours after inoculation sucrose was added to 14 of them, the leaves in the remaining 14 being left on the distilled water. From five to six days later uredinia appeared on practically all of the leaves, and at this time one-half of the leaves on sucrose were rinsed and placed on distilled water, and one-half of those on water were placed on sucrose.

Fifteen days after inoculation, teliospores were found in large numbers in the old uredinial pustules of those leaves which had been continuously on distilled water; also they were being formed on those leaves which had been transferred from the sugar to the water. In the latter case they were not at all numerous and the leaves were severely injured. Various results were obtained with the leaves which had been continuously on the sugar solution. Some of the leaves had teliospores and some died after producing the uredinial stage. In all cases where the leaves remained in good condition and the infection was not heavy, teliospores were formed.

On leaves which had been originally on water and were transferred to the sugar solution, most interesting phenomena were observed. The same sori which had originally produced urediniospores and later formed teliospores again formed urediniospores when the effect of the transfer to the sugar solution was felt. The later-formed urediniospores were not very numerous but sufficient to show that by the addition of the sucrose a new food supply had been provided for uredinial production. Thus we find the following spore forms being formed successively: urediniospores, as a result of the first infection; teliospores, as a result of the distilled water for the first eight days; and lastly, the second production of urediniospores as soon as the sugar solution had had an effect. It is interesting to note that some of the leaves which were producing urediniospores for the second time were completely brown: scarcely a trace of the original green color was to be seen.

As has already been explained, the time of year with its corresponding light intensity was a deciding factor with those leaves which were put on distilled water only, and which supplied to the rust only that food which was being formed in the leaves through photosynthesis carried on while the leaves were in the dishes.

Puccinia antirrhini Diet and Holw., on Antirrhinum majus L. Attempts were made to culture *Puccinia antirrhini* on Antirrhinum leaves on solutions, but infections were so scanty that reliable conclusions could not be drawn. The leaves themselves remained in good condition in the dishes for five or six weeks either on nutrient solutions or on distilled water. Occasionally adventitious rootlets were put out from the petiole and would grow until they attained lengths of ten centimeters or more. In one instance a rooting leaf was placed in a flask with Shive's solution and remained alive for 60 days; in attempting to induce it to grow in a pot of earth, decomposition set in and it was destroyed.

The leaves were finally infected on the lower surface, as has been explained elsewhere in the paper, and from then on good infections were always obtained.

As has been noted in the work with Antirrhinum rust in the greenhouse, the telial stage is rare under greenhouse conditions. Likewise in the dish cultures, the uredinial stage may continue to form for long periods of time on sugar solutions and the telial stage may never appear. On infected leaves on distilled water the pustules may dry up before more than just the primary uredinial pustules can be formed.

Early in the experimental work before a successful method of inoculating the leaves had been discovered, a few experiments were conducted with cuttings in solutions. Ten cuttings were inoculated, five of which were placed with the stems in flasks of distilled water and five in a 7 per cent solution of sucrose. They were kept during the whole of the experiments under bell jars to prevent excessive transpiration. Six days after inoculation, when the first signs of uredinial production became visible but before the pustules had broken through the epidermis of the leaves, three of the cuttings in sucrose were transferred to distilled water. The others were allowed to remain in the same solutions.

On the sixteenth day after inoculation, teliospores were found on the leaves of the cuttings which had been transferred from the sucrose to the distilled water. They were not numerous and were interspersed with urediniospores in the original uredinia. There were no teliospores on the leaves of the other cuttings. The pustules on the leaves of the cuttings in the distilled water were dried up as a result of rapid starvation or desiccation. On the cuttings in the sugar solution secondary uredinia were produced in abundance, but due to contamination it was impossible to determine whether teliospores would eventually have formed or not. While these results are not in themselves especially conclusive, when taken in conjunction with the results obtained later with detached leaves on solutions they show the relationship between the available food in the host and the production of teliospores.

In experiments with the detached leaves alone, it was found that leaves which were on sugar solutions continuously, if the amount of infection was not too heavy, usually produced the greatest abundance of teliospores. Rust on leaves which were transferred from sugar to distilled water, if the transfer was made before the fungus had advanced too far in its development, generally produced the telial stage.

Table 11 gives the results of some of the experiments conducted with *C. antirrhini* on detached leaves of *Antirrhinum*.

In experiment A, leaves which were on distilled water continuously did not form teliospores because, as has been found to be the case with all of the rusts, when the infection was heavy the rust famished so quickly that the production of teliospores was impossible. In Experiment B it is seen that when the infection was light teliospores were formed even though the

leaves were on distilled water all of the time. In Experiment C the same results were obtained as in Experiment A under the same conditions. The infection in the case of the leaves on distilled water was too heavy to allow teliospore formation. The other results were similar to those obtained with other rusts with the exception that when Antirrhinum leaves were transferred from distilled water to sugar solutions the leaves failed to take up the sugar. As a result, in Experiment A, where the leaves were transferred from water to sugar, the rust failed to respond to the change in environment and gradually died out without producing any more spores.

TABLE 11.—*The effect of different media on teliospore formation in Puccinia antirrhini on Antirrhinum majus*

No. dishes used	First nutrient	No. days on first nutrient	Second nutrient	Incubation time in days	Formation of teliospores (no. days after inoculation)
Experiment (A)					
6	Distilled H_2O	10	7% sucrose	9–10	None
5	do	10	Distilled H_2O	9–10	do
7	7% sucrose	10	do	9–10	19
7	do	10	7% sucrose	9–10	25
Experiment (B)					
8	Distilled H_2O	17	Distilled H_2O	8	17[a]
8	7% sucrose	21	7% sucrose	8	21
Experiment (C)					
12	Distilled H_2O	25	Distilled H_2O	10	None
14	7% sucrose	25	7% sucrose	8	25

[a] Infection very light; average no. of pustules per leaf about 20.

Puccinia sorghi Schw., on Zea mays L. *Puccinia sorghi* was readily cultured on leaves in petri dishes and, as Mains (21) reported, good infection could be obtained in the dark when the leaves were floated on sugar solutions.

Teliospores were induced in this rust by the same general means as in the other rusts already discussed. If sucrose was added at a time when the rust was beginning to feel the effects of the diminishing food supply, teliospores were formed. They were found on leaves which were on sucrose continuously, and also on leaves which were transferred from sucrose to distilled water before the uredinial pustules were fully formed. They were never produced on leaves floated on distilled water only. It was found that, if leaves were transferred from sucrose to distilled water after the uredinia were formed, teliospores were never formed; if, however, the

transfer was made three or four days after inoculation, the telial stage always appeared. The teliospores were in all cases interspersed at first with the urediniospores until finally there were only teliospores in the pustules.

Puccinia triticina Eriksson, on Triticum vulgare Vill. The experiments with *Puccinia triticina* were but repetitions of the ones with *P. sorghi*, for these two rusts and hosts seemed to behave similarly. That there is a difference, however, is evidenced by the fact that teliospores of *P. triticina* are seldom, if ever, seen on young plants of wheat in the greenhouse. Mains (22) says of this rust: "*Puccinia triticina* upon wheat seedlings has never been seen to produce telia under such conditions even on old dying leaves, but does produce some telia on old leaves when the wheat plants approach maturity." Teliospores were produced on a few young corn plants in the greenhouse but they were never induced on wheat.

On the leaves in dishes the writer has been able to produce teliospores with the same ease as on corn leaves.

To avoid the contamination that is so likely when culturing wheat leaves, leaves which were already infected on the plant were used. They were taken from the infected wheat plants before the uredinia had broken through and placed upon distilled water in the dishes. After three days, one-half of the leaves were placed upon the 7 per cent sucrose solution, the other half remaining upon distilled water.

Five days later an abundance of teliospores was found upon the leaves which had been transferred to sugar. They were formed in linear sori in most cases but some were interspersed with urediniospores in the old uredinia. There were many abnormal spores: some thick-walled, one-celled with spines; others two-celled with spines; and some like the typical two-celled, smooth-walled teliospore.

As was mentioned above, wheat and corn leaves cultured in dishes were more liable to contamination than any of the leaves belonging to the dicotyledonous group. Dicotyledonous leaves have been kept in dishes for several weeks with few signs of contamination, but corn and wheat leaves usually were covered in a few days with yeasts and other contaminating fungi.

This can probably be explained by the fact that in the monocotyledons there is usually an abundance of glucose and other monosaccharides stored within the leaf while in the dicotyledons there is little sugar stored as compared with the large amounts of starch (Parkin, 32). As the monosaccharides are easily attacked by the average saprophytic fungus, it is natural to expect such leaves to be contaminated sooner than those containing insoluble starches. In placing the leaves in dishes it is sometimes neces-

sary to cut the edges, and it is at these points that there is probably a diffusion outward of the stored sugars. These regions afford a favorable place of attack for the saprophytic forms.

Puccinia taraxaci Plowr., on Taraxacum officinale Weber. Taraxacum rust is easy to culture on leaves in dishes, but the formation of the spore type desired is very difficult to control. Uredinial formation continues sometimes up to the point where the whole leaf is destroyed. In many cultures upon sucrose solutions urediniospores continued to form for seven weeks, and even up to the point where only a small fragment of the leaf remained in the dish, new urediniospores were being formed.

The rust was grown upon both sugar solution and distilled water and the usual transfers made from one to the other, but with no results other than the continued production of urediniospores or the death of the leaves. Finally it was found that by transferring infected leaves from the sugar solution to distilled water and then back again, teliospores were produced.

The leaves were first inoculated in the usual manner on distilled water and, after two days to allow for spore germination, were transferred to sucrose solution. Here they remained for 16 days, in the meantime producing secondary rings of uredinia. They were then rinsed thoroughly and placed back again upon distilled water for five days, and then finally replaced upon the sucrose solution. Four days after the last transfer, examination revealed teliospores interspersed with urediniospores in some of the secondary sori. They continued to form for several days, but later urediniospores were formed instead. These continued to form for several days until the leaves became so contaminated that they were discarded. New sets of leaves were tried, and in each case teliospores made their appearance in practically the same manner and under the same type of manipulation.

The results of these experiments, although somewhat different from those for the other rusts, show the same relationship between food condition and production of teliospores. The host requires a further state of exhaustion before the telial stage is produced. That this is true can be determined from field observation on the nature of the host and the rust. A study of this rust over a period of three years in the field has failed to reveal a single case where the telial stage has been present except in the late summer or fall. This shows that the metabolism of the host and the fungus are so closely adjusted that the telial stage is produced under only the most well adjusted conditions. Magnus (20) has noted this situation in the Taraxacum rust and has explained it as being due only to the fact that the host is so hardy that its metabolism is not readily upset. Quite in contrast with this rust is that upon *Cirsium* where the least unfavorable condi-

tions for the host immediately throw the spore production of the parasite from the uredinial to the telial stage. A study of the life history and habits of these two hosts, however, seems to the writer to explain fully the differences in sensitiveness of the two. Both conditions are the result of a long period of evolutionary adaptation of each rust to its particular host. The same relationship exists in each case, and it is only when the experimenter has succeeded in finding the exact condition that upsets the perfect balance between host and parasite that the rust is stimulated to produce the resistant spore. The same principle holds true in each case, the only difference being the ability to simulate the proper conditions.

Puccinia orbicula Pk. & Clinton, on Prenanthes alba L. Perhaps the most striking demonstration of the effects of food conditions on the type of spore produced by the rust parasite was made with *Puccinia orbicula.* On the very young plants urediniospores and teliospores accompany the aecia quite profusely if the sorus occurs adjacent to the midrib or the larger veins. Generally upon the stems there is an almost pure teliospore production. The larger and more developed leaves contain a greater percentage of urediniospores than the younger. To all outward appearances this rust is somewhat similar to the Podophyllum rust in the distribution of the teliospores. Just as Whetzel, Jackson, and Mains (51) termed the latter "a plastic rust," so it appears that the Prenanthes rust might be regarded as quite plastic.

Healthy leaves of several ages were brought in from the field and infected with material obtained from rusted plants. Dishes P1–4 contained leaves which were about two-thirds mature floated on sucrose, the margins being cut to facilitate the absorption of the nutrient. Dishes P5–8 contained large, more matured leaves, but with only the petioles immersed in the sugar solution. Dishes P9–12 contained very young leaves on distilled water. Dishes P15–18 contained very young leaves with their petioles immersed in sucrose solution, and dishes P15–18 large leaves floated on distilled water.

Four days after inoculation, the large leaves with the cut margins had become thoroughly reddened due to the formation of anthocyanin, the leaves with only the petioles immersed becoming progressively red as the solution traveled up the veins.

On June 10, or 11 days after inoculation, urediniospores appeared on the leaves in dishes P1–5. The sori were very large and quite numerous. The same day they appeared on leaves P5–8, but were not so large nor so numerous. On the next day the uredinia appeared on the remaining leaves; in the case of P9–12 they were small, few in number, and contained about 90 per cent teliospores; P13–15 showed fair infection with many teliospores

FIG. 3. Rusted leaf of *Prenanthes alba* which had been transferred from distilled water to sucrose solution after having been on the distilled water for four weeks. New uredinia are seen breaking through the epidermis around the old telia.

accompanying the urediniospores. Leaves P15–18 had a few scattered sori, but in all cases they proved to be almost wholly filled with teliospores; only a very few urediniospores had formed.

Three days later, all of the leaves were producing teliospores. On the leaves in dishes P1–4 urediniospores were produced for the longest period; on the others teliospores were found in the same ratio as given above. The experiment was abandoned with the exception of dishes P15–18. The plants in these dishes were allowed to remain on distilled water for four weeks, and then placed on sugar solution. Four days after the transfer, examination revealed the surprising fact that the leaves had become decidedly red in color and new uredinial pustules were breaking through the epidermis (Fig. 3). The pustules could only have been the result of mycelia that had been dormant in the leaf for some time; and the sudden addition of the nutrient had renewed growth so that uredinia were produced. It must be remembered that these were the same leaves that four weeks earlier had shown only telial formation on the distilled water.

The dishes had remained closed for the preceding few weeks and there was little chance that the infections were the result of urediniospores from the outside. To the writer the logical conclusion appears to be that the type of spore which had been first produced and that which was later produced was determined wholly by the presence or absence of the carbohydrate within the cells of the leaf.

DISCUSSION

General Principles

It is evident that there exists between the host and its rust parasite a very close and intimate relationship, with the life processes of the two so closely attuned that a slight disturbance in the metabolism of the former is sufficient to disturb the latter and cause it to pass from a stage of active fructification, as evidenced by the uredinial generation, to one of quiescence, the telial. In extreme cases the uredinial stage may be entirely suppressed and then only the telial stage appears.

That the host does possess a very direct influence upon the fungus has been shown by Arthur (3), who points out very clearly that, as a rule, the vigor of the parasite is directly proportional to the vigor of the host; and that to be successful in culturing the rust fungus on its host it is necessary to use strong, rapidly growing plants. Stakman (41) believes that whatever favors the normal development of the host is ordinarily conducive to the vigorous development of the parasite. Ward (49) found that a starved host meant necessarily a starved rust, while Stakman and Levine (43) have shown that environmental conditions unfavorable to the host were also unfavorable to the rust. That the virulence of the disease may be directly

correlated with the "vegetative vigor" of the host has been pointed out by Raines (33). Sheldon (38) concluded that the lowered vitality of the host does not favor infection and anything affecting the growth of the host also affects in like manner the rust.

With the opinions and results of these investigators in mind, let us consider for a moment the source and nature of the food of the fungus, and see whether the above conclusions and those to follow do not show definitely that the rust fungus is dependent upon the photosynthetic activity of the host; and that any factor, or set of factors, such as temperature, light, moisture, etc., or a complex of these factors, such as climate, will so influence the metabolic condition of the host that it will be reflected in the type of spore produced by the rust. Until we can definitely separate the rust fungus from its obligate association with the host, and study the direct relationship of the fungus to such environmental factors as light, temperature, etc., while culturing it under strictly saprophytic conditions, we are not justified in assigning specific external influences to it. We are compelled to consider the rust fungus in terms of its most influential environment, viz., the host.

To determine the dependence of the rust fungus upon the photosynthetic activity of wheat seedlings inoculated with *Puccinia graminis*, experiments were made by Stakman and Levine (43). They concluded that "Inasmuch as the photosynthetic activities of the host plant are affected by the light intensity, in so much does the structure and function of the rust depend on the same factor."

That the rust is dependent upon intermediate products of photosynthesis was advocated by Fromme (12), while Mains (21) suggested that it is probably dependent upon some transitory or nascent organic products related to the carbohydrates.

Similar evidence of the dependence of the rust upon the photosynthetic activity of the host was obtained by the writer (50) in the case of bean rust, *Uromyces appendiculatus*, and in the present experimental work in ten additional species of rusts. It has been found in all the experiments conducted that once the parasite has gained entrance to a susceptible and congenial host, those conditions which guarantee an optimum supply of carbohydrate food for the host likewise insure a vigorous development of the fungus, as evidenced by a copious production of urediniospores. If, however, through the influence of internal or external factors, or a complex of such factors, the host is no longer able to maintain this condition of vegetative vigor, coincident with its ability to increase and maintain the carbohydrate content of its cells, then the fungus will, if still able to function, react to such conditions, and produce the resistant or telial stage.

Iwanoff (18), who attempted experimentally to prove the direct effect of climate upon certain rusts, illustrated this point in an irrefutable manner. He found that when he transported infected plants from regions of low altitudes to alpine stations he obtained a suppression of the uredinial stage with the hastening of the telial generation. He obtained the same results when he placed infected plants in an ice-chest in the regions of low altitude. The writer also produced the telial stage in seven species of rusts by the latter method. In the case of bean rust, when infected plants bearing uredinia were placed in a cold chamber at a temperature of 7° C. for two days, the telial stage appeared 13 days earlier than on the checks which remained in the greenhouse. Similar results were obtained in the other six species.

Morganthaler (28) transported plants of *Veratrum album* L., infected with *Uromyces veratri* (DC.) Schr., to alpine regions and found that the telial stage was produced sooner than in those plants which remained at low altitude.

Now, do any of these results justify the conclusion that the climate as such or the temperature in itself was directly influential in producing the telial stage? Is it logical to assume that the bean host represents the same set of food conditions at 7° C. as it did at 20° C? In a similar manner, can Iwanoff say that the same rate of metabolism was going on in his host plants at the plains station at an average temperature of 20° C. and an altitude of 520 meters, as was present at the alpine station Faulhorn at an altitude of 2684 meters and a temperature ranging from 2 to 28° C.? Morganthaler took account of these questions and concluded from his experiments that the climate was not directly responsible, but that the change in the altitudinal position of the host plants affected their nutrition, probably retarding it, and in this way induced the formation of the teliospores. He furthermore demonstrated that, by cutting the veins of leaves and by detaching or singeing leaves of *Veratrum album* infected with *Uromyces veratri*, he could induce the telial stage of the rust. He thus concludes that he is not far wrong in assuming that the principal cause for teliospore formation is the diminishing of food; that teliospores assume the role of resistant spores in the life history of the rusts and that the taking away of food stimulates their appearance.

Gassner (14) likewise was unwilling to ascribe to climatic influence the simple direct action that Iwanoff suggests. In his opinion the climate always acts upon the rust in a roundabout way through the host. He showed through extended observations and experiments that, under normal conditions of growth, the time of appearance of the telial stage of grain rusts has a certain relation with the stage of development of the host plant.

This usually occurs at the time of, or just preceding, the heading out of the grains, when there is a general mobilization of food within the plant for seed production. This stage he called the point of exhaustion of the host and assumed that at this stage the diminution of the food caused the fungus to produce the telial stage. He also showed that different hosts reacted differently in that stage of exhaustion. This was illustrated by *Puccinia triticina* and *P. graminis;* he succeeded in obtaining the uredinial stage of the latter upon hosts which were already bearing the telial stage of the former. It appears, however, that Gassner, although he was close to the fundamental issue of the matter, associated too closely the developmental stage of the host with telial production. Practically all his experimental work was done in the field, where environmental conditions other than purely climatic were not operative. In only two or three instances does he mention any attempt to use experimental means to hasten or retard telial formation. In the one case he cut notches in the leaves of the rusted hosts but with no results, the leaves dying soon after. The fungus on starved plants grown on quartz sand failed to produce the telial stage. By decreasing the light he did succeed in inducing the formation of teliospores on infected oat plants sooner than on the checks.

It would seem that even though, in the grain rusts, the telial stage usually accompanies a certain stage of development of the host, as Gassner has shown, it is only that the food situation at that time is in a state of mobilization for seed production and as a result the fungus is cut off from its food supply. The condition, under which the fungus suffers from nourishment inadequate for urediniospore production, may be brought about in a number of ways, so that the particular phase of the story brought out by Gassner is but one angle of the situation. The writer produced these same conditions by subjecting the infected hosts, without regard to age or stage of development, to low temperatures, lack of light, insufficient moisture, and by floating detached leaves upon various nutrient solutions. It is quite natural that under field conditions the telia would appear normally at a certain stage of development of the host; but the same host, under varying conditions of environment, may be so affected as to produce the telial stage at any or at all periods in its life history.

This is illustrated in the case of *Puccinia sorghi*. Gassner (14) found that on plants of *Zea mays* the telial stage appeared at about the time of flowering, *i.e.,* when the plants were about three or four months old. In the present paper it is reported that teliospores were produced on corn plants which were only 30 days old, and which were exposed to low temperatures for only one day. In this case it is evident that the stage of development of the host was not important.

The experiments described in the present paper are but a few among many that could be conducted to illustrate this fact. To bring about the proper balance of food supply between the fungus and the host to produce the telial stage is a problem that depends entirely on the experimental technique and that only continued trials will solve. This is seen in the cases of the different hosts used in the temperature experiments. In the behavior of the Cirsium and Taraxacum rusts the same principle is involved, with the same outcome in both cases; but to bring about the same results in the two hosts a different procedure is required. As mentioned previously, when Cirsium leaves were placed in the cold room for five days the telial stage was produced eight days sooner than on the checks. In the case of *Taraxacum*, placing the infected plant under the same conditions for seven days failed even to stop uredinial production. Three days in a similar environment completely killed the corn plant. Thus, it is evident that the structure and habit of the host plant must be taken into consideration as well as the environment to which it is accustomed. Having discovered the conditions under which the metabolism of the host can be regulated, it is safe to assume that the rust will correspondingly reflect the varied conditions in the type of spore which it will produce.

In attempting to interpret the results of the temperature experiments with such seemingly divergent rusts as those on *Taraxacum* and *Cirsium*, we must consider the nature of the two hosts as found under field conditions. *Cirsium* grows very rapidly: its life processes are carried through in exceedingly rapid succession. In three weeks a plant two or three inches in height may become several feet tall and have fully developed flowers. Under such conditions it is evident that there must be speedy assimilation and translocation of food. The rate of metabolism of the leaves becomes progressively slower as the tissues rapidly mature, and to a parasite inhabiting the leaves of such a plant the food becomes quickly unavailable. What is the result? As one would expect, either the fungus becomes adapted to such conditions and is capable of quickly protecting itself or else it is exterminated. Such protection comes through the formation of teliospores. These are produced at the first sign of waning metabolism, and by the time the food is completely diminished the telial stage is formed. Likewise, under experimental conditions, when the infected host is subjected to any abnormal environment retarding its metabolism, the rust will quickly respond by producing teliospores. Hence, when Cirsium leaves were placed under the influence of low temperature, absence of light, or when the detached leaves were transferred from sugar solution to distilled water, the results were identical with those obtained when the host approaches maturity in the field. Similarly, the telial generation is produced when leaves are placed continuously upon

distilled water if, on such leaves, there is a light infection. Ordinarily, on heavily infected leaves, which obtained no nutrient other than that which was synthesized within the dishes, no teliospores were produced. In other words, the fungus starved before telia could be formed. A similar case has been referred to by Gassner in answering the objections of Lagerheim, who maintained that the telial stage does not appear with the exhaustion of the host plant, as he had observed wilted plants of *Vicia faba* L. infected with *Uromyces fabae* de Bary in Quito, Ecuador, on which only urediniospores were visible. Gassner points out that this is probably due to the fact that the plants had wilted suddenly and were not able to reach maturity. He uses this example to point out that wilting and exhaustion are not identical. However, if Gassner had changed experimentally the environmental conditions of this same host he probably would have found that even as wilting and exhaustion are not identical conditions, neither is exhaustion of the host associated necessarily with its later or maturing stage of development.

In the case of the Taraxacum rust, where we find apparent differences in reaction, closer analysis reveals the same underlying influence of nutrition. Under the ordinary methods of greenhouse culture no teliospores were ever produced on the Taraxacum plant, for the uredinial stage persisted until the death of the infected host parts. But, when the host was subjected to successive exposures of low temperature, the telial generation made its appearance, as demonstrated in plants T10–15.

Taraxacum grows rather slowly in Michigan. The leaves are numerous and grouped closely upon a central axis, with no necessity for rapid assimilation or translocation of elaborated food over long distances. The plant may be found growing more or less actively in almost any month of the year. One would expect the food situation in such a plant to be stable, with no such rapid fluctuations as occur in *Cirsium*. In such a host the sudden diminution of available food, at least during the growing season, is not felt by the rust parasite. It is only with the advent of short days and low temperatures, when there is a gradual withdrawal of food from the leaves of the host, that the fungus is stimulated to produce the telial stage. The life processes of the host and fungus are so in accord that the latter is a direct result of the former. Any treatment that will bring about this unfavorable condition of food in the host will cause the fungus to produce the telial stage. Such a condition was produced when *Taraxacum* was subjected to alternate periods of low temperature and absence of light. Afterwards, when the host was returned to a favorable environment, the rust resumed its formation of the uredinial generation.

The same correlation between the type of host employed and the method

of manipulation required to produce the telial stage of the rust inhabiting such a host is seen in petri-dish culture.

In the case of *Cirsium*, teliospores appeared on the leaves in the dishes under several sets of conditions. If the leaves floated on distilled water were not too heavily infected, the telial stage appeared soon after the first appearance of the uredinia. Likewise, if the leaves were transferred from the 7 per cent sugar solution at the time of uredinial production to distilled water, the telial stage appeared. On the leaves which were continuously on sugar, providing the infection was not too heavy, teliospores formed also, but only after a longer period of time. It is possible that if the leaves could be so kept that they would continue to absorb the carbohydrate gradually, the uredinial stage would persist for much longer periods of time. In spite of all precautions, it is probable that the outer layers of cells adjacent to the cut surfaces die either as a result of bacterial action or autolysis, and further absorption ceases. In such cases, of course, telial formation occurs.

Another explanation which might be presented to explain this cessation of uredinial development on the leaves is that the rusts may not exist upon a purely elaborated carbohydrate diet but require some products related to the proteins. In such an event, when the leaf is left continuously upon the sugar solutions its cells would soon be depleted of such mineral nutrients as nitrogen, potassium, etc., and the further assimilation by the host of food suitable for the fungus would cease. Even the fact that the rust thrives for a time upon a leaf so nourished does not preclude the possibility of a partial protein-related diet. In an ordinary detached leaf there undoubtedly exists sufficient amounts of nitrogen, potassium, etc., within the cells to insure the normal development of the rust for a considerable period. The experiments of Ward (49) and Mains (21) showed that even when the host plant was starved with respect to certain mineral elements, infection could still take place.

To induce teliospore formation upon the leaves of *Taraxacum*, as is shown in the results of the experiment, it was necessary to make several changes in the medium upon which the leaves were floated. They were first floated upon distilled water, then immediately after inoculation changed to sugar solution for 16 days. At the end of this time they were transferred to distilled water for five days, after which they were returned to the sugar solution.

At the time of the transfer from sugar to distilled water the rust was producing an abundance of uredinia. This would mean that a great amount of food was being taken up from the sugar solution by the leaf and appropriated by the fungus. The sudden change to distilled water cut off this continuous food supply and stimulated the fungus to initiate the produc-

tion of the telial generation. However, due to the insufficient supply of stored food both in the leaf cells and in the mycelium, the fungus could not carry through to completion the formation of the telial stage. This was shown when the leaves were allowed to remain on distilled water at this stage of the experiment. If, after a period of five days the leaves were again transferred to the sugar solution, adjustments already initiated due to the stimulus were carried over and the fungus completed the formation of the telial stage. Soon after, the effects of the addition of the nutrient to the leaf cells again caused the fungus to resume uredinial production.

The Light Relations

With regard to the influence of light upon the growth and development of the rust mycelium, little need be said, for Fromme (12), Mains (21), and the writer (50) have shown that, in the organisms employed by them, the main influence is exerted indirectly through the host. We can consider that any results obtained from the experiments reported in this paper represent the effects of light acting not especially upon the fungus, but mainly and, perhaps only, through the host.

In five of the organisms studied, namely, *Uromyces appendiculatus*, *Puccinia suaveolens*, *U. trifolii*, *U. polygoni*, and *P. sorghi*, it was possible to inhibit the uredinial stage and hasten the time of telial formation by placing the infected plant in the dark for varying periods of time. The time of appearance of teliospores varied up to a certain limit directly with the length of time the host was left in darkness. Since the temperature was practically the same as for checks in the light, the effects produced could only have been due to the decrease in photosynthetic activity of the host, with the resulting partial starvation of the fungus.

It must not be assumed, however, that mere starvation and injury to the host in all cases will guarantee the appearance of the telial generation. It is undoubtedly true that the diminution of food will initiate the formation of such a spore stage, but in all cases the fungus must have access to enough nutrient to carry through to completion the reaction to such a stimulus. This was shown in the case of corn rust. When the host was subjected to total darkness for either three or four days, both host and parasite died before the telial generation was formed. Two days, however, under the same conditions produced the necessary "starvation" stimulus and, when the host was again removed into the light to the greenhouse, it was capable of synthesizing just enough food to enable the fungus to complete the formation of the telial stage. If the metabolism of the host and fungus are not too greatly disturbed, it should be possible, in some cases, to induce uredinial formation again following the telial stage. This is what actually occurred in the case of *Uromyces polygoni*. When plants

P1–5 and P5–10 were removed from the cold, dark room (7° C.), and the dark room (19° C.), respectively, after having been there for four days, the telial stage was first produced; then, under the influence of the favorable conditions of the greenhouse, the uredinial generation was again produced. When, for the second time, the plants were subjected to the same conditions of light and temperature as before, the fungus proceeded to produce the telial stage again. This time, however, plants P1–5 were not able to respond to the resumed favorable conditions and the fungus continued to produce the telial stage until the death of the infected leaves. On P5–10, which had not been so severely affected, uredinial production was resumed for a short time before finally the telial stage appeared.

Several of the other rusts reacted in a similar fashion, and the results appear so positive that discussion is not deemed necessary to point out further the direct connection between the presence or absence of food and the type of spore produced.

Moisture Relations

The experiments conducted relative to moisture control seem to show that, in certain rusts at least, the amount of moisture available for the host and parasite, be it soil or atmospheric moisture, exerts an influence on the type of spore produced by the rust parasite.

The conclusions drawn by the writer from the results of experiments conducted with asparagus rust are perfectly in accord with those of Smith (40), as far as the assumption goes that teliospores are produced when moisture conditions, be it soil or atmospheric, are unfavorable for the further vegetative development of the fungus. The lack of agreement comes in the interpretation of the manner in which the moisture affects the fungus.

Smith assumes that an abundance of soil moisture favors the host by giving it increased vitality and resistance with the result that the fungus is retarded and forced to form the telial stage. This is clearly at variance with the findings of such workers as Arthur (3), Stakman (41), Ward (48), Stakman and Levine (43), *et al,* mentioned at the beginning of the discussion. However, as Smith's conclusions are based on field observations, it appears to the writer that there are too many unknown factors which may have entered in to ensure the absolute accuracy of his interpretations.

Sheldon (37, 38), was forced to disagree with Smith's interpretations, for he concluded that, as a rule, conditions favorable for the development of the host were also favorable for the development of the fungus. The same conclusions were reached by the writer, with an added feeling of assurance that an abundance of soil moisture not only increases the vitality of the host but also lengthens the period of uredinial production by the rust.

When plants A5–6 were placed in the greenhouse and kept well watered they continued to produce the uredinial stage for a week longer than did plants A1–5, which were deprived of their soil moisture.

If the conclusions of Smith be correct, then the presence of abundant soil moisture should have rendered the host an unfavorable medium for the fungus and the telial stage should have appeared sooner than on plants A1–5. This, however, was not the case. On the contrary, the results seemed to show that the abundance of soil moisture exerted a favorable effect on the host and also on the fungus, due to the increased ability of the former to produce an abundance of food.

With respect to the action of atmospheric moisture, Smith (40) asserts that "atmospheric dryness checks aecidial development and uredo-development, and changes to a production of teleutospores in the sori already formed, without regard to season or conditions of the host plant. With moisture, uredospore formation begins again at once."

It is difficult to understand how such a statement can be positively made, for such an assertion disclaims any influence that the living host might exert at the same time. Unless Smith can show that the asparagus rust parasite fails to maintain that close association with the host which is common to so many of the rusts, he must admit that the condition of the host should play an exceedingly important rôle. Can he maintain that the host, under the influence of high humidity, is, physiologically speaking, the same host upon which the telial stage was being produced? Is it not true that the increase in humidity would tend to lessen the transpiration and evaporation from the infected stalks with the result that the metabolic rates of both the fungus and the host would remain lower and the cells of the host remain alive for a longer period of time? Would not this in itself affect the food relations of the fungus?

Resistance of the Host

Fromme and Wingard (13) found a relationship between the resistant varieties of bean and teliospore formation. When certain resistant varieties of bean were inoculated, teliospores, or mixed sori, made their appearance immediately.

In working with *Puccinia coronata* Corda, on *Avena sativa* L., Parker (31) observed that frequently the telial stage appeared on seedling leaves of resistant varieties, while on the susceptible varieties it did not occur.

In table 3 it is seen that on the Scarlet Wax variety of bean, which is resistant to rust, teliospores were formed 15 days after inoculation, or only three days after the first appearance of the uredinial stage. In some cases the teliospores appeared without being preceded by this stage.

It is impossible to analyze all the possible factors in resistant and susceptible plants, as no one has yet satisfactorily explained what the phenomenon of resistance actually means. The cytology of infection, both in resistant and susceptible plants, has been investigated by several, among whom are Ward (47, 48), Evans (11), Stakman (41, 42), and Allen (2). In all cases they have shown that, when a rust infects a host, vigorous development takes place without any immediate serious injury to the host. In fact, the host cells seem to be stimulated to greater activity for a while. In resistant hosts, however, the host cells in the immediate vicinity of the haustoria are killed and the fungus makes little headway. The extent to which this killing takes place seems to depend upon the degree of resistance of the host. Whether, as Marrayat (24) has declared in the case of *Puccinia glumarum*, the fungus starves as a result of the death of the host cells, it is difficult to say; but, at any rate, from all the cytological evidence presented, it is certain that the fungus in a resistant host is not able to establish a satisfactory food relationship with the host. Such being the case, it is entirely possible that this condition of partial starvation would immediately stimulate the production of teliospores as was seen in the bean.

Since, as has been pointed out in all the above experiments, the appearance of the telial stage is an index of some metabolic disturbance within the host, it is entirely within reason to suppose that the early appearance of the telial stage on a resistant plant is due to the inability of the fungus to establish a satisfactory food relationship with the host.

SUMMARY

1. From the results of the experiments herein described, it appears that all the rusts studied are directly dependent upon the photosynthetic activity of the host. Any single factor or set of factors, such as light, temperature, and moisture, or, as in the case of climate, a complex of these factors, may so influence and do influence the metabolism of the host, that the fungus reacts by changing from the uredinial to the telial generation, or, under proper manipulation, in the reverse direction.

Results obtained in the greenhouse

2. Nine species of rusts were cultivated in the greenhouse and experiments conducted to show that teliospore production can be controlled.

3. With the exception of *Puccinia triticina* on *Triticum vulgare*, teliospores were formed in all the rusts when the host plants were placed under various environmental conditions unfavorable for their metabolism.

4. In seven of the rusts, teliospores were produced when the infected hosts were placed in darkness at 7° C., or in darkness at 19° C.; in the

former environment teliospores appeared several days sooner than in the latter.

5. The conditions of light and temperature necessary for production of the telial stage vary with different hosts. Such conditions appear to be governed largely by the nature and habits of growth of the host.

6. In three of the rusts, teliospores were produced when the infected hosts were slowly deprived of water, the amount and general procedure varying with the nature and habit of the host.

7. Resistance of the host plays a part in the production of teliospores. In the case of bean rust (when the host was partially resistant) the telial stage was formed several days earlier than when it was susceptible. In some cases the uredinial generation was completely inhibited and teliospores were the first structures to appear following infection.

8. By removing the growing point of the bean plants immediately following inoculation and thus preventing the withdrawal of food from the primordial leaves, the time of teliospore formation was postponed several days.

Results obtained in petri dishes

9. A method was devised by which detached leaves or portions of leaves of the host plant could be infected and floated on water or nutrient solutions in petri dishes. By this method infections were obtained regularly and in some cases they exceeded in amount these obtained on plants in the greenhouse.

10. Ten rusts, including the nine used on potted plants, have been cultured by the above method. The additional rust was *Puccinia orbicula* Pk. and Clint. on *Prenanthes alba* L.

11. Urediniospores and teliospores were produced in eight of the rusts which were cultured in dishes, the type of spore formed depending on the treatment to which the leaves of each host were subjected.

12. The rusts in which the telial generation was produced most easily in the greenhouse were also the ones which responded most readily to experimental methods in dishes.

13. When infected bean leaves were transferred at the time of uredinial formation from a 7 per cent cane-sugar solution to distilled water, if the infection were not too heavy, teliospores were produced much sooner than on leaves which were allowed to remain on the sugar solution continuously.

14. On infected bean leaves, grown on distilled water, teliospores appeared greatly in advance of those on leaves which were better nourished.

15. The heaviest infection on bean leaves was obtained by transferring the leaves from distilled water to sugar solution six days after inoculation.

16. Starch tests made on bean leaves at the time of uredinial and telial formation showed an abundance of starch at the time of uredinial formation and a paucity of starch during the production of telia.

17. In the case of most of the rusts cultured in petri-dishes, teliospore formation was stimulated by any one of the three following methods: (a) starvation of the host with the later addition of a rich food supply, (b) sudden transfer of the host leaves from a well-fed condition to a state of starvation, (c) continuous supply of food with the gradual dying of the host cells.

18. In two of the rusts the telial generation was induced only by transferring the leaves from the sucrose solution to distilled water and back again.

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LITERATURE CITED

1. ACTON, E. H. The assimilation of carbon by green plants from certain organic compounds. Proc. Roy. Soc. London **47**: 150–175. 1889.
2. ALLEN, RUTH. Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici* forms III and XIX. Jour. Agr. Res. **26**: 571–604. 1923.
3. ARTHUR, J. C. Problems in the study of plant rusts. Bul. Torrey Bot. Club **30**: 1–18. 1903.
4. ————. Terminology of the spore-structures in the Uredinales. Bot. Gaz. **39**: 219–222. 1905.
5. ATKINS, W. R. G. Researches in Plant Physiology. 328 pp. New York. 1916.
6. BOEHM, J. Ueber Stärkebildung aus Zucker. Bot. Zeit. **41**: 32–38, 49–54. 1883.
7. CARLETON, M. A. Culture methods with Uredineae. Jour. Appl. Micr. and Lab. Meth. **6**: 2109–2114. 1903.
8. CLINTON, G. P., and F. A. MCCORMICK. Rust infection of leaves in petri-dishes. Conn. Agr. Exp. Sta. Bul. 260. 1924.
9. CZAPEK, F. Der Kohlenhydrat-Stoffwechsel der Laubblätter im Winter. Ber. Deut. Bot. Gesell. **19**: 120–127. 1901.
10. DORAN, W. L. Rust of *Antirrhinum*. Mass. Agr. Exp. Sta. Bul. 202. 1921.
11. EVANS, J. E. P. The cereal rusts. I. The development of their uredo mycelia. Ann. Bot. **21**: 441–466. 1907.
12. FROMME, F. D. The culture of cereal rusts in the greenhouse. Bul. Torrey Bot. Club **40**: 501–521. 1913.
13. ————, and S. A. WINGARD. Varietal susceptibility of beans to rust. Jour. Agr. Res. **21**: 385–404. 1921.
14. GASSNER, GUSTAV. Die Teleutosporenbildung der Getreiderostpilze und ihre Bedingungen. Ztschr. Bot. **7**: 65–120. 1915.
15. HACKEY, J. F. Germination of teliospores of *Puccinia antirrhini*. 13th Ann. Rept. Quebec Soc. Protect. Plants. 54–57. 1921.
16. HENNINGS, P. Anpassungsverhältnisse der Uredineen bezüglich der physikalischen Beschaffenheit des Substrates. Hedwigia. **40**: 125–128. 1901.
17. HUNT, N. REX. The "iceless refrigerator" as an inoculation chamber. Phytopath. **9**: 211–212. 1919.

18. IWANOFF, B. Untersuchungen über den Einfluss des Standortes auf den Entwickelungsgang und den Peridienbau der Uredineen. Centralbl. Bakteriol. Abt. II. **18**: 265–274. 1907.

19. LIDFORSS, B. Zur Physiologie und Biologie der wintergrünen Flora. Bot. Centralbl. **68**: 33–44. 1896.

20. MAGNUS, P. Ueber das Auftreten der Stylosporen bei den Uredineen. Ber. Deut. Bot. Gesell. **9**: 85–92. 1891.

21. MAINS, E. B. The relation of some rusts to the physiology of their hosts. Amer. Jour. Bot. **4**: 179–220. 1917.

22. ————————. Notes on greenhouse culture methods used in rust investigations. Reprint from Proc. Ind. Acad. Sci. **33**: 241–257. 1923.

23. ————————. Notes on the life history of the snapdragon rust, *Puccinia antirrhini* Diet. & Holw. Phytopath. **14**: 281–287. 1924.

24. MARRYAT, DOROTHEA. Notes on the infection and histology of two wheats immune to the attacks of *Puccinia glumarum*, yellow rust. Jour. Agr. Sci. **2**: 129–138. 1907.

25. MELCHERS, L. E. A way of obtaining an abundance of large uredinia from artificial culture. Phytopath. **5**: 236–237. 1915.

26. MELHUS, I. E. Culturing of parasitic fungi on the living host. Phytopath. **2**: 197–203. 1912.

27. MEYER, ARTHUR. Bildung der Stärkekörner in den Laubblättern aus Zuckerarten, Mannit und Glycerin. Bot. Zeit. **44**: 81–88, 105–113, 129–137, 145–150. 1886.

28. MORGENTHALER, O. Ueber die Bedingungen der Teleutosporenbildung bei den Uredineen. Centralbl. Bakt. Abt. II. **27**: 73–92. 1910.

29. MUDGE, C. S. The effect of sterilization upon sugars in culture media. Jour. Bact. **2**: 403–415. 1917.

30. OLIVE, E. W. Intermingling of perennial sporophytic and gametophytic generations in *Puccinia podophylli, P. obtegens* and *Uromyces glycyrrhizae*. Ann. Mycol. **11**: 295–311. 1913.

31. PARKER, J. H. Greenhouse experiments on rust resistance of oat varieties. U. S. Dept. Agr. Bul. 629. 1918.

32. PARKIN, JOHN. Contribution to our knowledge of the formation, storage and depletion of carbohydrates in monocotyledons. Trans. Roy. Soc. London. Series B. **195**: 35–79. 1899.

33. RAINES, M. A. Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants. Amer. Jour. Bot. **9**: 183–203, 215–238. 1922.

34. ROSTRUP, E. Ein eigenthümliches Generationsverhältniss bei *Puccinia suaveolens* (Pers.) Rostr. Bot. Zeit. **32**: 556–557. 1874.

35. SAPOSCHNIKOFF, W. Die Stärkebildung aus Zucker in den Laubblättern. Ber. Deut. Bot. Gesell. **7**: 258–260. 1889.

36. ————————. Ueber die Grenzen der Anhäufung der Kohlenhydrate in den Blättern der Weinrebe unter anderer Pflanzen. Ber. Deut. Bot. Gesell. **9**: 293–300. 1891.

37. SHELDON, J. L. Preliminary studies on the rusts of the asparagus and carnation. Science **16**: 235–237. 1902.

38. ————————. The effect of different soils on the development of the carnation rust. Bot. Gaz. **40**: 225–229. 1905.

39. SHIVE, J. W. A three salt nutrient solution. Amer. Jour. Bot. **2**: 157–160. 1915.

40. SMITH, R. E. Water relation of *Puccinia asparagi*. Bot. Gaz. **38**: 19–34. 1904.

41. STAKMAN, E. C. A study in cereal rusts; physiological races. Minn. Agr. Exp. Sta. Bul. 138. 1914.

42. ————. Relation between *Puccinia graminis* and plants highly resistant to its attack. Jour. Agr. Res. **4**: 193–200. 1915.

43. ————, and M. N. LEVINE. Effect of certain ecological factors on the morphology of the urediniospores of *Puccinia graminis*. Jour. Agr. Res. **16**: 43–77. 1919.

44. TOLLENAAR, DIRK. Omzettingen van Koolhydraten in het Blad van *Nicotiana tabacum* mit Referat: Der Kohlenhydratstoffwechsel im Laubblatt von *Nicotiana tabacum*. Het Laboratorium voor Landbouw-Scheikunde en het Laboratorium voor Plantenphysiologisch Onderzoek. No. 12. 1925.

45. TUBEUF, K. F. VON. Diseases of plants. English Edition. 598 pp. London, New York, and Bombay. 1897.

46. WARD, H. M. Illustrations of the structure and life history of *Puccinia graminis*, the fungus causing the "rust" of wheat. Ann. Bot. **2**: 207–222. 1888.

47. ————. The Bromes and their rust fungus (*Puccinia dispersa*). Ann. Bot. **15**: 560–562. 1901.

48. ————. On the relations between host and parasite in the Bromes and their brown rust, *Puccinia dispersa* (Erikss.). Ann. Bot. **16**: 233–315. 1902.

49. ————. Experiments on the effect of mineral starvation on the parasitism of the uredine fungus, *Puccinia dispersa*, on species of *Bromus*. Proc. Roy. Soc. London **71**: 138–151. 1902.

50. WATERS, C. W. The reaction of bean rust grown on leaves in solutions. Papers Mich. Acad. Sci. **5**: 163–177. 1925.

51. WHETZEL, H. H., H. S. JACKSON, and E. B. MAINS. Composite life history of *Puccinia podophylli* Schw. Jour. Agr. Res. **30**: 65–79. 1925.

A MOSAIC DISEASE OF GLADIOLUS[1]

LOUISE DOSDALL

While examining Gladiolus corms for various diseases in 1925 several Primulinus hybrid corms were found with a peculiar deformed appearance suggesting cucumber nubbin. The normal corm in this variety is plump with a smooth, clear, yellow epidermis, whereas the abnormal corms were distinctly warty and the color was mottled. On the elevated areas the epidermis was yellowish, while in the depressed areas it was paler and sometimes greenish.

Several of these corms were planted in pots in the greenhouse, together with several normal corms. Certain symptoms quite characteristic of mosaic diseases developed in the plants from the peculiar corms. The leaves pushed out to about one-half their normal length, then ceased to develop further. They seemed somewhat thicker and stiffer than normal leaves and were mottled much like sugar cane leaves affected with mosaic. The plants from the normal corms were taller, the leaves were dark green, and there was no mottling. An attempt was made to produce the disease in healthy plants by extracting the juice from mottled leaves and rubbing it on the surface of uninjured young growing leaves and on young leaves which had been pricked, and by injecting the juice with a needle. Only negative results were obtained.

In the summer of 1925 the grower from whom the corms were obtained had reported that, while his plants seemed to be free from disease, there was a very decided difference in the appearance of the plants from his old corms and those from new corms which he had obtained from the east. With the same variety there was a sharp line marking where the one set of plants stopped in the row and the others began. The new plants were much darker green and seemed more vigorous than the old ones.

In the spring of 1926, while going over a lot of miscellaneous corms, many of the peculiarly warted ones were noted. Fifty of these warted corms were selected. As a check, fifty smooth corms as nearly similar in size as it was possible to select them were taken. Both lots were treated with formaldehyde and planted in parallel rows. Ninety-four per cent of the smooth corms and only seventy per cent of the checks produced plants. The plants from the warty corms were decidedly less vigorous than the others, and nearly all of the plants developed the same type of mottling

[1] Published with the approval of the Director as Paper No. 732 of the Journal series of the Minnesota Agricultural Experiment Station.

in the leaves as did the plants which had grown in the greenhouse. Some of the plants in the check row also developed mottled leaves. This mottling of the leaves was found to be extremely common in all gladiola plantings visited during the summer.

The plants from the warty corms blossomed considerably earlier than the others. The first blossom opened on July 12 in the one case and on July 26 in the other. Of the plants from warty corms, 48 per cent produced blossoms; of the checks, 95 per cent. Most of the plants in this experiment belonged to the variety Gretchen Zang. In many cases the mottling was very prominent on the peduncle and bracts of the inflorescence and extended also into the petals (Pl. II, A). The normal pink color of the petals was broken with greenish white.

There was a decided difference in the yield of corms from the two lots. Very few cormels were produced by the diseased plants and the corms were only one half the size of those from the check row. Both lots of corms were again planted in parallel rows in 1927. Fourteen per cent of the progeny of the original selection of warty corms produced plants. Fifty-six per cent of the progeny of the original check produced plants. Practically all of the plants in the diseased row were mottled. Most of the plants in the other row also showed signs of the disease to a greater or less extent. The second year (1927) only one plant in the diseased row blossomed. This plant proved to belong to the variety Willie Wigman. The leaves were dark green and there was no evidence of mottling.

The same type of disease has been observed in various varieties. In the 1926 planting of Pride of Goshen, many of the plants were badly stunted, the leaves were mottled, and the malformations of the inflorescence were pronounced. The peduncle was very much shortened, the individual flowers were closer together and smaller than the normal, and the petals were almost greenish. Twenty-six corms from the planting were replanted in 1927. Of these, only seven produced plants. Only one of the seven plants produced a normal blossom. A planting of the variety Alice Tiplady next the Pride of Goshen in 1926 was particularly vigorous and no signs of the mosaic were noted. When the corms were cleaned during the winter, however, the buds under the sheaths were found to be covered with the cotton aphid, *Aphis gossypii*. In 1927 many of the plants from these corms were mottled. The symptoms on the leaves were particularly striking (Pl. II, B). This is a Primulinus Hybrid type with flowers far apart on the central axis. On plants in which the infection extended into the blossom the shortening of the peduncle and bunching of the flowers in the inflorescence was very marked. The breaking of the color in the blossom was very pronounced also. Aphids were abundant on the plants until the

first leaves were about half grown. Later in the season none could be found. Plants of the variety Schwaben have also been badly infected. The malformation of the corm is very marked (Pl. II, C), as is also the stunting of the plants and the mottling of the leaves and flowers (Pl. III, A).

The peculiar wartiness of the corms is very marked in the variety Gretchen Zang (Pl. II, D). On cutting sections through such warty corms it was found that the abnormality in structure extended through the entire corm. From freehand sections it was found that this structure was due to the fact that there were areas of large cells filled with starch grains and areas of small cells with no starch grains quite similar to the green and chlorotic areas in mosaic infected leaves. In the normal corm the starch is distributed evenly throughout the corm. This difference is shown very beautifully by staining slices of corms with iodine (Pl. II, E). Similar results were obtained with Schwaben and Primulinus Hybrid corms.

These observations indicate quite definitely that the abnormal condition is a degeneration disease of Gladiolus. The symptoms are very marked at all stages in the life of the plant; on the corm, the leaves, and the inflorescence; and are characteristic of the symptoms of mosaic diseases in other plants. The disease is transmitted through the corm from one generation to the next. Eventually the plant is killed. Probably the disease is transferred from one plant to another by sucking insects although this has not yet been demonstrated to be true.

UNIVERSITY FARM,
SAINT PAUL, MINNESOTA.

EXPLANATION OF PLATES

PLATE II

A. Inflorescence of the variety Gretchen Zang showing the breaking of the color in the blossom.

B, a. A diseased leaf of the variety Alice Tiplady showing the characteristic mottling.
b. A normal leaf of the variety Alice Tiplady.

C, a. A diseased corm of the variety Schwaben.
b. A normal corm of the variety Schwaben.

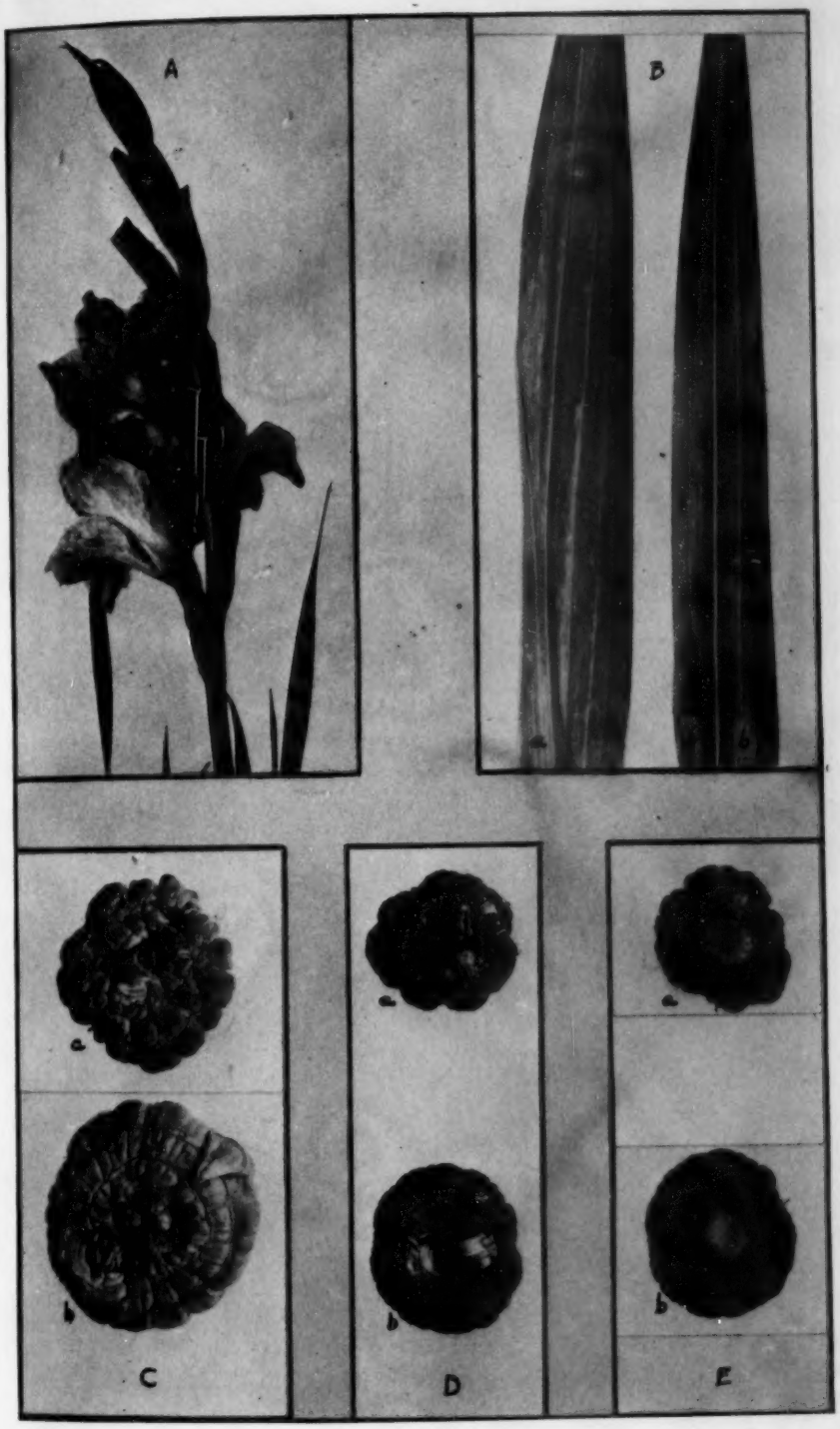
D, a. A diseased corm of the variety Gretchen Zang.
b. A normal corm of the variety Gretchen Zang.

E, a. A section of a diseased corm of the variety Gretchen Zang, stained with iodine.
b. A section of a normal corm of the variety Gretchen Zang, stained with iodine.

PLATE III

A. Inflorescence of the variety Schwaben showing the characteristic mottling on the peduncle and bracts and to some extent the crowding of the flowers.

B. A normal inflorescence of the variety Schwaben.



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WASHINGTONIA PALM LEAF SPOT DUE TO CYLINDROCLADIUM MACROSPORUM N. SP.

C. D. Sherbakoff

INTRODUCTION

In September, 1916, the writer found in a glass-covered bed, in central Florida, seedling leaves of *Washingtonia robosta* Parish. severely affected with numerous, small, dark leaf spots with translucent borders. On the spots was found a Hyphomycete which was readily recognized as belonging to Morgan's[1] genus *Cylindrocladium* and very similar, except for a marked difference in the size of the conidia, to his *C. scoparium*, the fungus found on a dead pod of *Gleditschia triacanthus*. The species was the only one known at that time.

At the end of 1916 the writer found that Dr. L. M. Massey[2] was working with a *Cylindrocladium* isolated from crown canker of roses and exchanged cultures with him. The conclusions of the writer's examination (Fig. 1, D) of Massey's rose fungus agree with his, that it should be considered the same as Morgan's *C. scoparium*. Dr. Massey also found that Ellis and Everhart's[3] fungus, found on dead leaves of the papaw tree, and described under the name of *Diplocladium cylindrosporium* E. and E., is also *C. scoparium*. In 1918 Dr. P. J. Anderson[4] published his work on rose canker, agreeing with Massey's conclusions regarding the identity of the fungus. However, in association with *C. scoparium* he found also another fungus of the genus and, because of its much smaller conidia and of some other differences, described it as a new species, *C. parvum*.

In 1919 Professor H. E. Stevens, then plant pathologist of the Florida Agricultural Experiment Station, collected some specimens of leaf spot on mature Washingtonia palm growing on the Station grounds and, because he thought that the spots might be similar to those previously found, gave them to the writer. Examination showed that the fungus was in all main characters, except for the size of conidia, exactly like the fungus found on the seedling leaves in 1916.

[1] Morgan, A. P. Two new genera of Hyphomycetes. Bot. Gaz. 17: 190–192. 1892.

[2] Massey, L. M. The crown canker of the rose. Phytopath. 7: 408–417. 1917.

[3] Ellis, J. and M. B. Everhart. New species of fungi from various localities, with notes on some published species. Bul. Tor. Bot. Club 27: 58. 1900.

[4] Anderson, P. J. Rose canker and its control. Mass. Agr. Exp. Sta. Bul. 183: 9–46. 1918.

In 1920 the writer left Florida and thus had no opportunity to complete the work with the fungi. However, in Tennessee, in 1922, he isolated a *Cylindrocladium* from the crown of a yellowish plant of red clover brought in by Earl Felix, a student. The fungus was found to be the same as *C. scoparium* in general cultural and morphologic characters and in size of the conidia. Finally, in 1924 and 1925 the writer isolated a *Cylindrocladium* from dark brown spots on specimens of roots of apple seedlings received from a western state. The apple-root fungus also appears to be *C. scoparium* (Fig. 1, E).

Representative members of the genus *Cylindrocladium* were thus found on a wide range of hosts covering a large territory, so that the fungi can hardly be considered as rare. But evidently they are usually overlooked by mycologists and plant pathologists. To call their attention to the subject is the purpose of this brief paper, which, in its present state, is merely a series of notes. In spite of the writer's wish to round up the subject and present it in a more finished form, it became apparent that the paper should be published without further delay, because the writer finds that he will not have sufficient time in the near future to give to the subject.

DESCRIPTION AND ECONOMIC IMPORTANCE OF THE WASHINGTONIA PALM LEAF SPOT

The spot is from less than ½ mm. to over 2 mm. in diameter, round to oblong, mostly with smooth though sometimes angular outlines, dark greenish-brown, with a narrow translucent border. The surface is smooth or, in damp air becomes covered with a thin, whitish, somewhat coarsely powdered growth of the fungus. The spots are about the same in appearance (Fig. 1) whether they occur on the simple leaves of the palm seedling or on the fan-shaped leaves of a mature palm, although in the particular specimens of the fungus collected from seedlings in 1916 and from the mature palm leaves in 1919 there were noticeable differences in size of the conidia.

The writer's observations indicate that the disease spreads rapidly and causes noticeable damage to the leaves only when there is abundant moisture in the air; otherwise the disease remains almost inactive for quite a long time and thus seems to be of no economic importance even in Florida's rainy season in the summer. The only time the disease was observed to do severe damage was in a glass-covered bed filled with palm seedlings where the ventilation was very poor.

The Fungus

A. P. Morgan's descriptions of the new genus *Cylindrocladium* and of the new species *C. scoparium* are as follows:

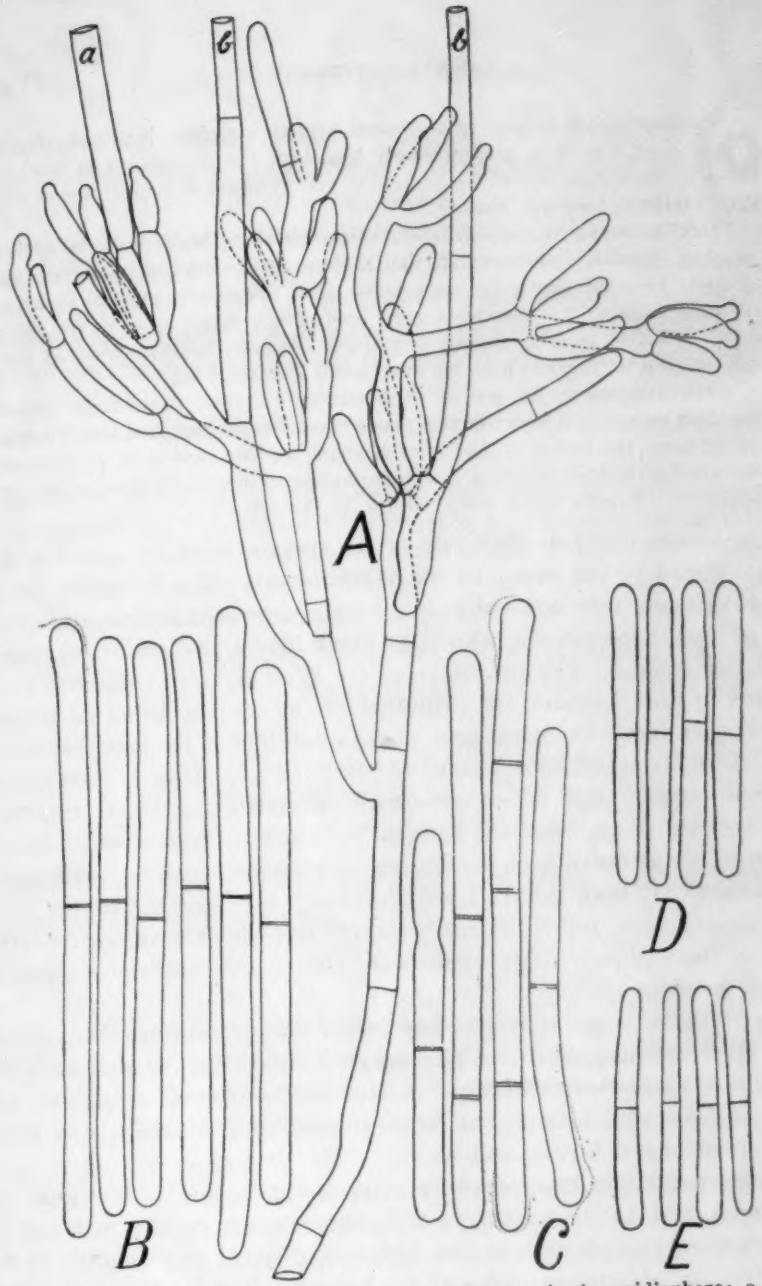

FIG. 1. A–C. *Cylindrocladium macrosporum* n. sp.: A. A conidiophore; a. Lower part of the conidium still attached to the sterigma; b. Lower part of long terminal hyphae which extend farther up from the point at the top of the drawing to from 100 to 200μ and are slightly swollen at the upper end; B. Typical conidia; C. Conidia showing deviations from the type either in the shape or in the septation; the dotted lines about some of the conidia in B and C show the visible lines of a water soluble substance with which the conidia are covered. D. Conidia of *C. scoparium* Morgan, from Massey's culture of the rose canker fungus, on cornmeal agar 14–day old plate. E. Conidia of *C. scoparium* Morgan, from the author's isolation from root spot of apple seedling, a young tube culture on potato agar. A–D are from camera lucida drawings; E is a reconstruction from a photomicrograph. Magnification 750 times.

"*Cylindrocladium* gen. nov.—Sterile hyphae creeping, branched; fertile hyphae erect, forked or trichotomously branched, the sporophores in pairs or threes at the extremities of the branchlets and cymosely arranged; spores solitary, cylindric, 1-septate, hyaline.

"*C. scoparium* n. sp.—Effused, thin, flocculose, white; sterile hyphae creeping, slender, indistinct; fertile hyphae thick, erect, hyaline, septate, cymosely branched above, the sporophores short, disposed in pairs or threes at the extremities of the branchlets, each producing a single spore at the apex; spores cylindric, tapering slightly downward, 1-septate, hyaline, obtuse at each end, 40–50 μ in length, 4 μ at the apex and 3 μ at the base.

"Growing on an old pod of *Gleditschia triacanthus*. The sterile hyphae abundant enough, but they are fine, slender threads creeping close to or beneath the surface; the fertile hyphae have a simple septate stem 5–7μ in thickness and are dissolved above into a level-topped cyme of branches; their height, exclusive of the spores which easily fall off, is 125–150μ."

The description and the figure of the fungus, when compared with the fungus found by the writer on the Washingtonia palm leaf spots, indicate that the two are very much alike in all main morphologic characters, except for the size of the conidia, which are much longer and somewhat wider in the latter fungus. The fungus from the Washingtonia palm was readily isolated in pure cultures, by a dilution of the conidia found on the spots and by planting in cornmeal agar plates small bits of the leaf tissues taken from the spots, which were washed for 10 to 12 seconds in 1 : 1000 mercuric chloride solution and rinsed afterward in sterile water. It may be of some interest to note that the fungus for a few months after its isolation produced an abundance of conidia on cornmeal and potato agars and on steam-sterilized bean pods. Later, however, the fungus lost the ability to produce spores, and it became necessary to renew the vigor of a culture by host inoculation. After reisolation, the fungus sporulated again for several months.

In addition to the conspicuously larger size of conidia, there are also some other morphological and physiological characters, as well as in relationship to the host, in which the fungus differs from *C. scoparium*, sufficiently, the author believes, to be considered as a separate, new species which is described here as follows:

Cylindrocladium macrosporum n. sp.—Fig. 1, A–C. Mycelium, conidiophores, and conidia hyaline; conidiophores erect, usually with one, two or more side branches each ending with two, three or more slightly curved, cyme-forming sterigmata; some of the branches, usually terminal, instead of producing sterigmata give rise to very long hyphae slightly swollen at the tip; on the sterigmata are produced singly long, cylindric, straight, obtuse, often slightly swollen at the ends, somewhat narrower at the base, 1-septate conidia which are apparently glued together with a water soluble, colorless substance so that when placed in water they easily separate. The

conidia in strain 1, from seedling palm leaves, measure 103.80 × 5.35 μ (71.00–131.00 × 5.15–5.94 μ), and those in strain 2, from leaf spot of mature palm, measure 82.00 × 5.25 μ (60.00–105.00 × 4.50–6.20 μ). The fungus in culture, especially in old cultures, usually produces chlamydospores in clusters and chains and some minute, submerged, brown sclerotia, though the latter were never observed to be so numerous and dark as in *C. scoparium*. The color of the species, on the same media, is noticeably lighter than that of *C. scoparium* and is free from the red color of the latter species observed in some cultures.

Habitat.—Found in Florida on leaves of *Washingtonia robosta*, causing leaf spots.

The size of the conidia of the different isolations of *C. scoparium* and of the species of the *Cylindrocladium* from the Washingtonia palm is as follows:

C. scoparium from *Gleditschia triacanthus*, 40–50 × 3–4 μ, (Morgan).

from *Asinina triloba*, 40–50 × 4–5 μ, (Ellis and Everhart).

from *Rosa sp.*, 48.30 × 4.13 μ (36–55 × 3.3–4.51 μ), (Massey).

from *Rosa sp.*, 39.20–48.80 × 4.03–5.10 μ, (Anderson).

from *Malus sp.*, 42.5 × 4.2 μ, (Sherbakoff).

Average size of conidia of *C. scoparium*.....45.0 × 4.2 μ.

C. macrosporum from *Washingtonia robosta*, strain 1 from seedlings:

40 conidia from 11-day culture on cornmeal agar plate—102.00 × 5.20 μ (75.0–125.0 × 4.9–6.0 μ). Measured in 1916.

20 conidia from old bean-pod culture—102.40 × 4.90 μ (92.0–118.0 × 4.5–5.2 μ). Measured in 1916.

100 conidia from 12-day culture on cornmeal agar plate—102.00 × 5.20 μ (74.0–123.0 × 4.9–5.5 μ). Measured in March, 1917.

40 conidia from 11-day culture on cornmeal agar tube—103.80 × 5.35 μ (71.00–131.00 × 5.15–5.94 μ). Conidia about 5.25 μ wide at narrow end and 5.47 μ at broad end. Measured in October, 1917.

Average size of the conidia of strain 1—102.50 × 5.20 μ.

C. macrosporum from *Washingtonia robosta*, strain 2 from mature leaves:

50 conidia from leaves—84.00 × 5.25 μ (60.00–105.0 × 4.5–6.2 μ). Measured October, 1919.

10 conidia from 5-day culture on cornmeal agar plate—78 μ (75–80 μ) in length. Measured in November, 1919.

Average size of the conidia of strain 2—83.00 × 5.25 μ.

Briefly the average size of the conidia of different Cylindrocladia known at present are: *C. parvum* about 17.00 × 2.50 μ; *C. scoparium* about 45.00 × 4.20 μ; *C. macrosporum*, strain 1—102.50 × 5.20 μ, strain 2—83.00 × 5.25 μ.

PATHOGENICITY

Massey and also Anderson definitely proved the ability of *C. scoparium* to produce the cankers on the greenhouse roses and showed the economic importance of the disease and devised certain methods of its control.

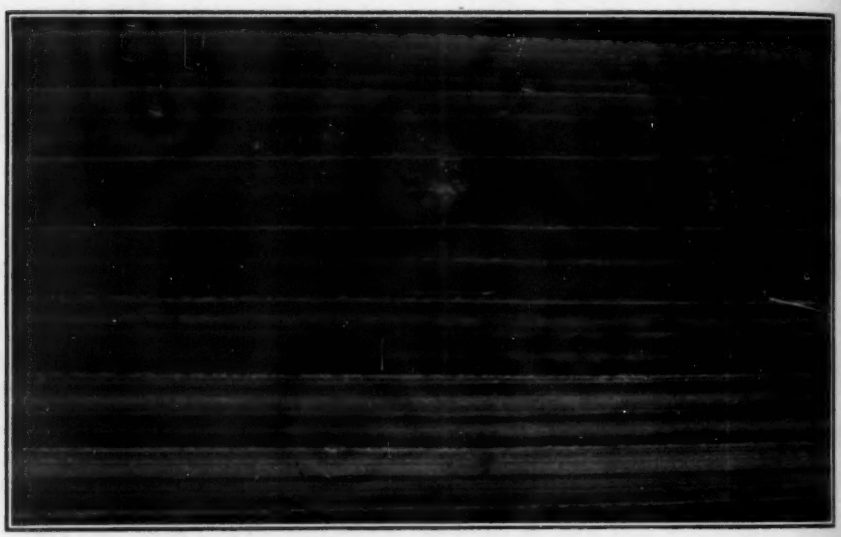

FIG. 2. A photograph of a part of the Washingtonia palm seedling leaf, showing the spots produced by an inoculation with a pure culture of *Cylindrocladium macrosporum*. The large spot—toward reader's left and slightly upper side of the photo—is where a bit of the inoculum was placed; the other spots arose evidently from the infections by the conidia scattered from the culture. About 1½ times natural size.

The writer, in March, 1917, made a few inoculations of the Washingtonia palm potted seedlings. The seedling leaves were sprayed with sterile water and inoculated with small bits of pure fungous cultures with many conidia from cornmeal agar. They were then covered with bell jars. For checks, several other palm plants were watered and likewise covered but not inoculated. Four days later, on the leaves of about half of the plants,

there appeared spots similar to those originally found (Fig. 2) and from which the fungus was isolated. Small bits of the tissues from the spots were then cut out, disinfected 10–12 seconds in 1:1000 solution of corrosive sublimate, rinsed in sterile water, and plated on cornmeal agar. On the plates pure growths of the *Cylindrocladium* developed, which in all characters, size included, were identical with the originally isolated fungus.

Acknowledgment. The work during 1916–1920 was done at the Florida Agricultural Experiment Station, Gainesville, Florida, where the illustrations, except part E, fig. 1, also were made. The paper is published with the permission of the Director, Wilmon Newell.

TENNESSEE AGRICULTURAL EXPERIMENT STATION,
KNOXVILLE, TENNESSEE.

CONSTANT TEMPERATURE AND HUMIDITY CHAMBERS

JAMES JOHNSON[1]

Six years ago the writer described a set of air-control chambers suitable for conducting experiments on the relation of environment to plant disease. Shortly afterward the chambers were moved to another greenhouse to secure refrigeration and at the same time several modifications were made which proved to be decided improvements on the earlier apparatus. These chambers have now been in almost continuous operation for five years with no modification and few repairs and are consequently deemed worthy of description for the benefit of those who contemplate the construction of similar apparatus.

The importance of studies on the relation of temperature and humidity to plant disease is fully recognized. The initiation of such investigations is largely limited by the difficulty of constructing equipment giving satisfactory control and by the cost of such equipment. It seems quite apparent, however, judging by the number of pathologists and physiologists expressing interest in air-control chambers, that the problem of controlled environment in relation to plants will receive increasing attention in the future. Since the construction of the chambers in question, two other units have been set up in the Wisconsin laboratories (2) (10), which are, however, more or less different from the type herein described. Air-control units for the growing of plants are also being used in several other American institutions, notably the Illinois Agricultural Experiment Station (3) (4), the Boyce Thompson Institute for Plant Research (1), and the Nebraska Agricultural Experiment Station (8).

GENERAL PLAN OF EQUIPMENT

The three chambers of the air-control series to be described are each located in separate but contiguous greenhouse units in which the temperature is thermostatically regulated to correspond roughly to the temperatures of the respective chambers which they contain. The purpose of this arrangement is to eliminate condensation of moisture on the glass walls of the chambers, which may occur when large differences in temperature exist between the surfaces exposed in very humid air. This arrangment also aids in reducing the amount of necessary refrigeration or heating, as the case may be, inside the chambers.

[1] Wisconsin Agricultural Experiment Station in cooperation with the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

The three chambers are identical in construction, except for the amount of refrigeration area contained in each. The chamber usually used for the medium degrees of temperature contains approximately 24 linear feet of one and one-half inch galvanized iron pipe. The chamber usually used for the lower temperatures contains 48 feet of pipe, which is in two units however, so that only 24 feet need be used if desired. The high temperature chamber can be operated with 27 feet, 24 feet or 3 feet of refrigeration pipe as desired (Fig. 1). The refrigeration can, of course, be cut off completely from any one or all of the chambers. The variation in length of refrigeration pipe is not only useful in permitting the use of wide ranges of temperature, but is important also in meeting the influence of seasonal conditions on the temperature inside the greenhouses. It is practically impossible even with a large refrigeration surface to combat the highest temperatures of summer. The chambers are therefore not ordinarily operated during the summer months for plant cultures, but with the sunlight shut out we have operated them for other purposes, such as the study of environment on the curing of tobacco. With the present system of refrigeration, constant temperatures as low as 10° C. can usually be secured during eight months of the year in a chamber containing 48 feet of refrigeration pipe.

The refrigeration in the chambers is furnished in the present instance by a 10-ton Audiffren machine (General Electric Company). This machine furnishes refrigeration for several other purposes, and a considerably smaller machine would, of course, suffice for the operation of the amount of refrigeration surface described. Refrigeration is such an important item of cost in the construction of chambers that it should be stated that the amount required is dependent upon a number of factors. Much can be accomplished in the way of constant temperatures during the winter months in the northern climates without any refrigeration. Where temperatures below about 18° C. are required, however, over a considerable period of time, artificial refrigeration is necessary. At mid-day, with strong sunlight, shading the chambers is often necessary, even with refrigeration, to hold the temperature down to normal, and this practice needs to be considered particularly in chambers lacking required refrigeration capacity. As it is ordinarily true that the critical temperature for plant diseases lies above 20° C., it may suffice in many cases to have refrigeration in only one chamber. For this purpose, a small machine of the type used for household refrigerators may sometimes prove sufficient. In general, the refrigeration system necessary will depend upon the prevailing temperatures at the season of the year at which it is desired to operate the chambers, and upon the host or the disease which is to be studied.

The supply of humid air for the chambers is furnished by a spray of water heated to the desired temperature. Since a spray of water operates continually for each chamber, it is important to have an adequate and continuous supply of warm water. In our units, this is furnished by a 60-gallon tank, in the water-pressure circuit, heated with high-pressure steam and regulated by a thermostat. The Instanto Steam Water Heater (North Manchester, Ind.) has proved very satisfactory for our purposes. This system also furnishes water for other air-control units, and a tank of half this capacity would be sufficient for the operation of the chambers described in this paper. Before high-pressure steam was available for this purpose, a 40-gallon, gas-heated water tank furnished the hot-water supply in a satisfactory but less reliable manner.

The refrigeration system and hot-water tank are located in a building adjoining the greenhouses through which the brine and hot water are piped to the chambers.

It is also important to install a separate line of electric current of sufficient capacity from the fuse box to the chambers, upon which no additional electric load can be added. The overloading of the electric line is likely to cause interruptions in the best operation of the control units. The heaters and motors of the chambers to be described require a line carrying approximately 60 amperes of electricity. All the apparatus in our chambers is operated with 110 volt, single phase, 60 cycle alternating current.

CONSTRUCTION OF THE CHAMBERS

The chambers are four-foot cubes with three sides and bottom of wood and the top and one side of glass (Fig. 1). This arrangement naturally sacrifices a considerable amount of light which might otherwise be available if the top and all sides were of glass construction. In actual practice, however, there does not seem to be any decided advantage in an all glass chamber. The normal illumination is greatly reduced in either case, and during periods of strong sunlight the shading is necessarily in proportion to the amount of glass surface exposed. There is no reason, however, why the air-control apparatus described for these chambers would not be equally applicable to chambers constructed with glass walls. The more satisfactory way of increasing the illumination of air-control chambers is by the use of artificial light, with a flowing water screen to absorb the heat from the lamps. The illumination is still far from "normal," however, and chambers may therefore be said to be useful only within certain limits. For our purposes we have found it preferable to rely on natural light, as usually only one to three weeks' exposure of plants in the chambers is found necessary.

The chambers are constructed of three-fourths inch cypress lumber, the bottom and three sides being double walled, with a dead air space and build-

ing paper between. The top and one side are constructed of double-walled, single-strength glass, inserted in door frames on hinges, which are held closed by ordinary window fasteners. The glass side in all chambers faces

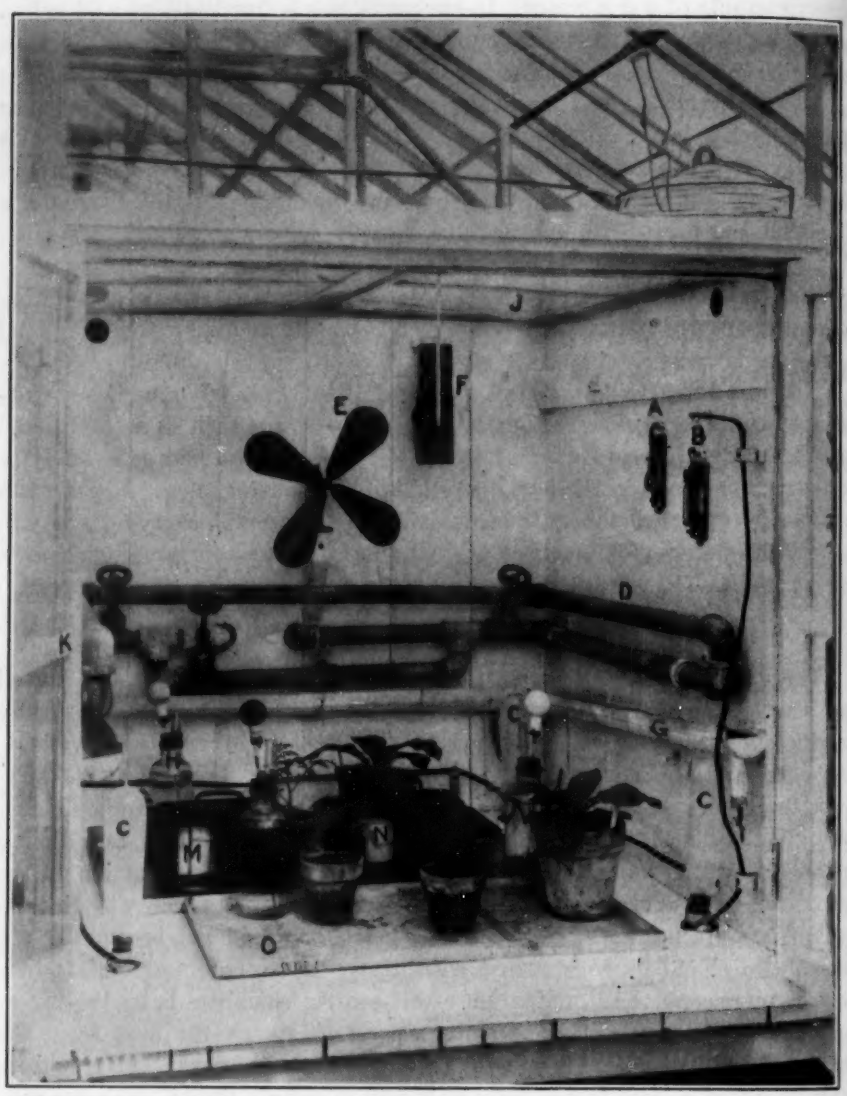

FIG. 1.—*Interior view of one of the air-control chambers.* A, electric thermostat; B, electric humidostat; C, heaters; D, brine pipes from refrigeration system; E, fan, driven by motor outside chamber; F, inlet for humidified air; G, drain for drip from brine pipes; H, atmometers; J, glass-top in door-frames; K, front glass door; L, plank bench; M, thermograph; N, hygrograph; O, drain pan for potted plants.

the south in order to obtain the maximum sunlight. The chambers are also kept painted pure white, which aids in the reflection of light within the chambers. The sides enclosed with glass also have wooden doors which can be attached when light is not desired in the chambers. Each chamber, with its apparatus, rests on a heavily constructed bench about two feet in height. This bench is four by six feet in area, so that four by two feet extend out to one side of the chamber upon which the humidifier "spray-tank" is placed, together with other required apparatus (Fig. 2).

TEMPERATURE REGULATION

The refrigeration system in the chambers has already been referred to. The heaters are of sufficient capacity to maintain a constant temperature against the refrigeration used. The practice is, therefore, to use only the approximate refrigeration surface necessary to hold the temperature down to the required degree during periods of the day when the highest temperatures occur.

Similarly, sufficient heating capacity must be available during periods when the temperature is likely to be at the minimum in the chambers. For our purposes, we have found two or three 250-watt Westinghouse Radiator units to be sufficient (Fig. 1, C). The heating units are placed in an upright position in the corners of the chamber. These heaters are regulated by a Johnson Service Company,[2] Number 2, electric thermostat (Fig. 1, A), which operates through a relay made by the same company (Fig. 2, F). By means of a transformer on the relay, the 110-volt current is reduced for the thermostat; consequently no batteries for this current are needed, greatly reducing the inconvenience, and adding to the reliability of operation. Aside from cleaning the contact points on the thermostats and relays two or three times a year, this system has required no special attention. The temperature can ordinarily be controlled to within one or two degrees (Fig. 3). During days of strong sunlight, in the spring and summer months, the temperatures are likely to rise unless the glass walls of the chambers are shaded, for which purpose we have used a thin white shade, conveniently hung above the chamber on an ordinary shade-roller so that the shade may be readily raised or lowered as desired.

HUMIDITY REGULATION

The source of the humid air consists of an air current passing through a spray of water held at a temperature a few degrees above that of the chamber to be humidified. The hot-water supply for all chambers flows from the steam-heated and thermostated tank which has been already referred to. The hot and cold water are mixed if necessary to secure the desired temperature. This can be accomplished by merely regulating the amount of

[2] Milwaukee, Wisconsin.

flow of hot and cold water in the pipe before it reaches the spray-nozzle. For this purpose it is important to have both sources of water on the same pressure line and to use valves which will maintain a constant position when set. The temperature of the water need not be carefully regulated, but for convenience in regulation it is advisable to insert a mercury thermometer beyond the "mixer" or into the spray-tank in a convenient position for reading. If the water used contains some solid material, it is well to pass it through a large screen somewhere in the main water line before it reaches the ordinary mist type of fruit tree spray nozzle which should also contain a screen. The "spray-tank" (Fig. 2, A) used is an upright cylinder, 15 inches in diameter and 5 feet high, in which the spray is confined. The top is a removable cover from which the spray nozzle, which is placed in a vertical position, can be reached for cleaning. At the base of the tank, a drain pipe carries the excess water to the sewer. About one foot above the base of the tank is a four-inch aperture, connected with an air-blower, and between the tank and the blower is a two-way or double-mixing damper enclosed with galvanized iron (Fig. 2, B). Through the humidostat, this damper regulates the passage of the humid air into the chamber. A short three-inch pipe carries the air from the upper end of the spray-tank into the chamber (Fig. 1, F). The blower (Fig. 2, C) used is a Number 0 "Sirroco" motor-driven blower (1/20 H. P.) which operates continually. In our earlier chambers the blower was operated intermittently by the humidostat, but this method was found to be much more injurious to the motors than continual operation. The action of the humidostat is therefore transferred through a special relay (Fig. 2, D) to the electro-magnet or automatic switch (Fig. 2, E) which operates the dampers, turning in or shutting off the humid air as required. The humidostat used is of the electrical contact type, manufactured by the Johnson Service Company, as is the relay, automatic switch, and six-inch double-mixing damper. The dampers can be operated, of course, by any solenoid of sufficient capacity, but we have used the automatic switches, which were originally used on the motors, as these were available, and have found them very satisfactory for this purpose. The covering of the mixing damper and the small levers which connect the automatic switch with the damper are not furnished with the apparatus and must be constructed to fit the particular arrangement used.

With this system of humidity control, the water spray and motors are in continuous operation. The air of the chambers is ordinarily held constant to within about five per cent of relative humidity (Fig. 4) by means of the control apparatus when properly adjusted. There is no circulation of air from the chambers back through the motors and tank, although this could be easily arranged if desired, by connecting the blower and the double mix-

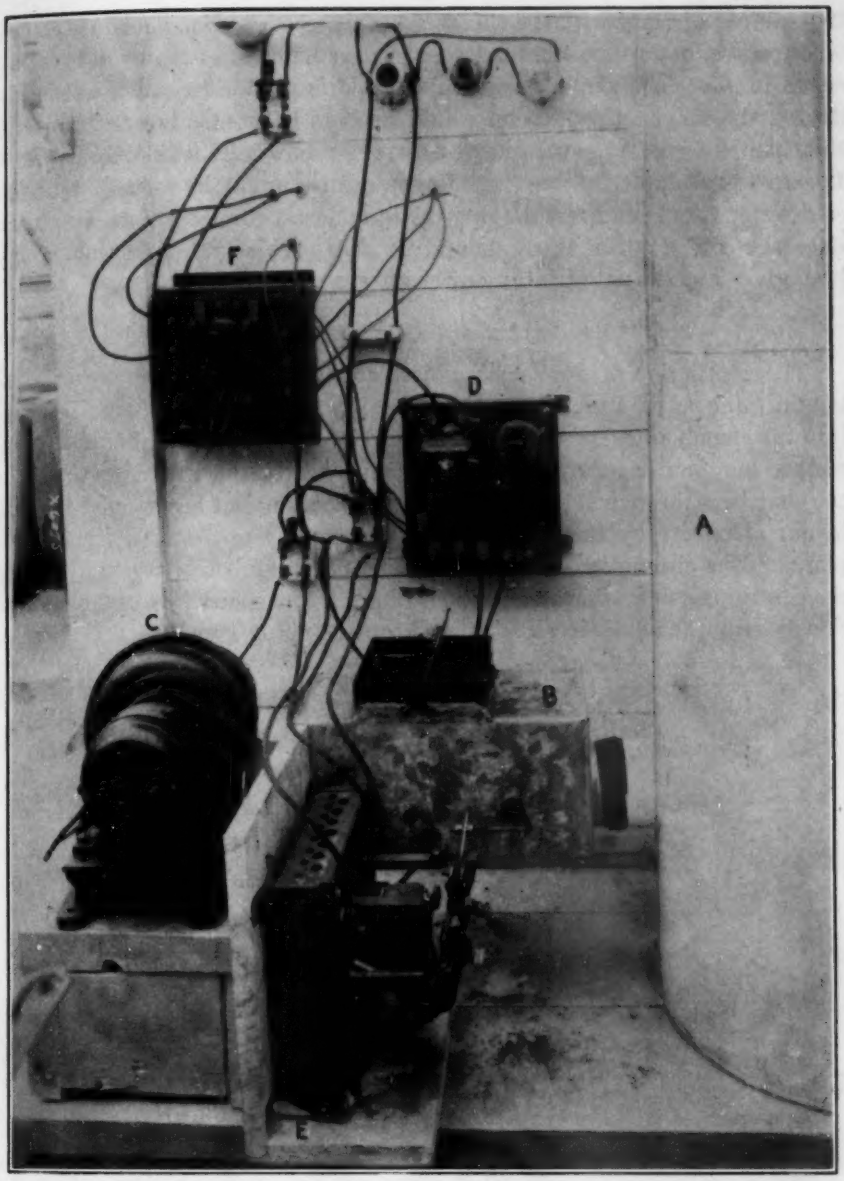

FIG. 2.—*Exterior view of one side of air-control chamber where practically all the control equipment is located.* A, spray-tank; B, "double-mixing" damper enclosed in galvanized iron box; C, blower; D, relay connected to humidostat; E, automatic switch operating damper; F, relay from thermostat operating heaters. Wiring is according to directions furnished with instruments.

ing damper directly with the chamber. This method of air moisture control is, of course, not applicable for lower relative humidities than will normally occur in the chambers without any humidification. In other words, for drying the air, another system would need to be used. Lower humidities than can be secured in such chambers are not, however, ordinarily required. It seems probable that constant low humidities might be secured by using cold water sprays and rearranging the humidostat connection so as to introduce the cold dry air as the humidity rises. This system has not as yet been given an adequate trial in our chambers.

VENTILATION AND CIRCULATION OF AIR

The ventilation of the chambers is accomplished essentially through the humidifying apparatus. Normally, new air from the greenhouse is blown into the chambers every few minutes by the blower as humidified air. This method may not have any important advantage over recirculating the air in the chambers through the blower for humidification, but has seemed justified in our chambers by the assurance that there was no foul air around the plants. The circulation of the air within each chamber is accomplished by the continuous operation of a 13-inch fan at a low speed (about 200 revolutions a minute) which is sufficient to keep the air in gentle motion (Fig. 1,

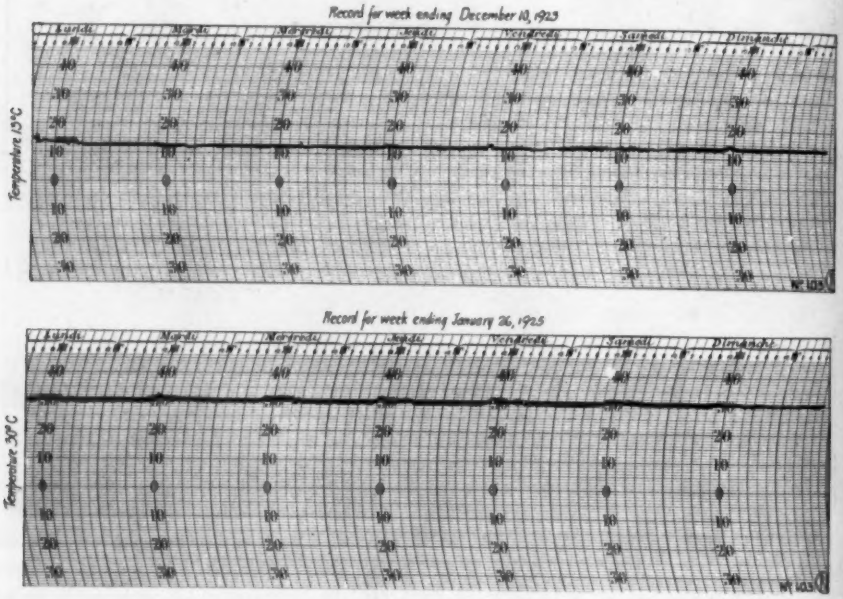

FIG. 3.—Thermograph charts illustrating the approximate constancy of temperature secured within the air-control chambers over a period of one week. Note that most of the disturbances occur near midday as a consequence of the influence of direct sunlight.

E). The fan is operated from a small pulley shaft extending through the wall of the chamber, where the shaft is driven by a belt from a small motor resting on a shelf attached to the wall of the chamber. The speed of the fan is therefore determined by the size and arrangement of the pulleys. It seems important to have some such arrangement, because motors operating in the interior of the chamber under conditions of high humidity are likely to be damaged.

RECORDING APPARATUS

It is desirable, if not necessary, to have the proper recording apparatus in each chamber for the records of temperature and humidity. The actual temperature in the chambers is determined from a suspended thermometer, but a thermograph (Fig. 1, M) shows at a glance whether or not the temperature has been constant throughout the week. The hygrograph (Fig. 1, N) is useful, but is not to be regarded as reliable for the actual percentage of relative humidity. For actual relative humidity we have relied upon the use of wet and dry bulb thermometers set in front of a rapid current of air, such as the Friez aspirating psychrometer.

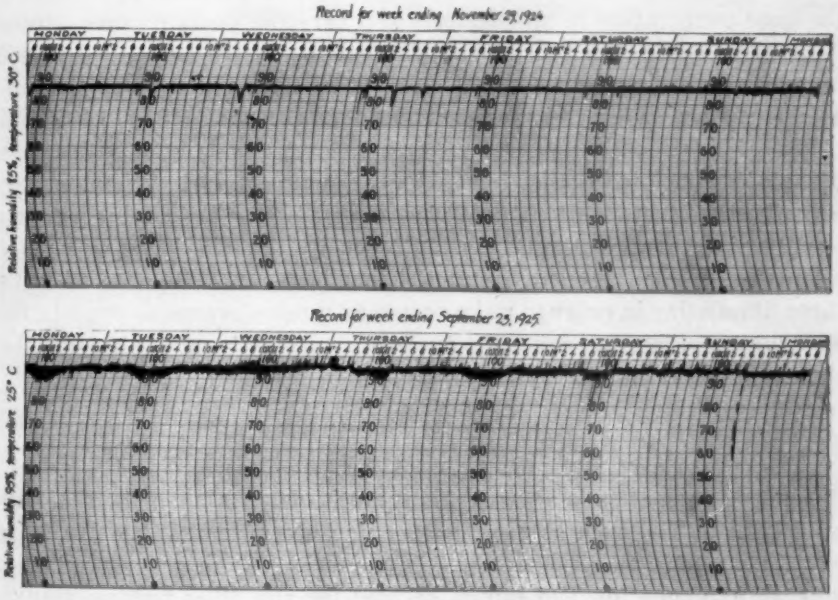

FIG. 4.—Hygrograph charts illustrating the approximate constancy of relative humidity secured within the air-control chambers. Humidity is more difficult to control satisfactorily and unless the instruments are carefully adjusted and kept in good repair much greater variations than shown in these graphs will occur. While the equipment is not infallible, the accuracy obtainable is believed to be sufficient for all ordinary purposes.

The evaporating power of the air naturally varies with the temperature, at any given relative humidity. We have usually planned to regulate the chambers according to the evaporating power of the air rather than according to the relative humidity. For this purpose, we have used three Livingston atmometers in each chamber (Fig. 1, H), the average of which is taken as a measure of the moisture conditions of the air in the chambers.

During one series of experiments, the records show that we have secured an atmosphere in the chambers with an evaporation of only 3.5 cc. a day (approx. 95 per cent rel. hum.) at a temperature of 91° F., as compared with an evaporation of 31.4 cc. a day (approx. 53 per cent rel. hum.) at 75° F. Low humidities with high temperatures and high humidities with low temperatures are, of course, more easily secured than high temperatures with high humidities and low temperatures with low humidities.

The temperatures at which the chambers can be operated are roughly within the range of plant growth. Temperatures as low as 10° C. and as high as 45° C. may be secured. Relative humidities as low as 30 per cent can be secured at the higher temperatures as well as high humidity at most temperatures desired. It is possible, therefore, to maintain approximately the same temperature in all chambers and vary the humidity or *vice versa*.

A great deal of the constancy and reliability of the air-control depends upon the careful adjustment of the instruments and maintaining them in a good condition. Poor electrical contact points account for most of the difficulties of operation. It is preferable to give regular daily attention to the oiling of the motors in order to avoid any danger of overlooking it entirely. Some types of motors are no doubt better than others for continuous operation, but in general they will run continuously for many years with proper attention. While the operation of the chambers should be watched two or three times a day in order to make necessary repairs as soon as possible, they may often operate satisfactorily for weeks with little more attention than the oiling of the motors.

COST OF CONSTRUCTION AND EQUIPMENT

The cost of air-control chambers of the type described in this paper is difficult to present satisfactorily, since much depends upon the equipment already available, such as greenhouse space, steam, running water, drain, electric current, and refrigeration. If part or all of this general equipment must be arranged for, and cannot be used for any other purpose than the operation of the air-control chambers, the total cost of construction will be relatively high. In the case of most institutions planning to erect air-control chambers, such general equipment and conveniences are already available and will not be considered in connection with cost of construction or operation of the air-control chambers. The following is an approximate estimate

of the cost of construction and equipment of one chamber. The carpenter work, material, and painting of the chamber ($4' \times 4' \times 4'$) and bench ($4' \times 6'$) as described may not cost over $60.00. The electric control equipment, including thermostat, humidostat, with relays for each, one automatic starter switch, and one six-inch double-mixing damper, will amount to about $125.00. A small motor and shaft with pulleys to operate fan, spray-tank, nozzle, valves, and piping for water and brine and heaters, together with other necessary small equipment, will cost about $75.00. The hygrograph, thermograph, and Livingston atmometers for one chamber cost approximately $200.00. Since the latter are not a necessary part of the chambers, and may be used for other purposes as well, they should probably not be charged to the cost of construction of the chambers. Excluding these instruments and assuming the necessary electric current, hot and cold running water, drain and refrigeration are available close at hand, the total cost of constructing one air-control chamber of the type described in this paper will be approximately $260.00. As three chambers operating simultaneously are usually necessary for satisfactory experimental work, the cost must at once be tripled. Such an estimate is, of course, only useful in giving those who are considering construction of similar apparatus an idea of the funds required.

EXPERIMENTAL RESULTS

It is not the purpose of the present paper to discuss in detail the results secured with these chambers. These have, for the most part, been reported elsewhere. In the first contribution (5) it was shown that the optimal temperature for the development of ordinary tobacco mosaic lies between 28° and 30° C. and that the maximum temperature lies at about 36°–37° C., at which temperature the symptoms tend to be masked. It was also shown that the optimal temperature for the wildfire disease of tobacco (*Bacterium tabacum*) is relatively high (28°–32° C.) and that the minimum is below 15° C. and the maximum above 37° C. The optimal temperature for the development of late blight of potatoes (*Phytopthora infestans*), after infection occurred, was found to be relatively high (25°–32° C.). While the critical temperature for the bacterial wilt of the tomato (*B. solanacearum*) was not determined, one experiment showed, for instance, a much more rapid progress of the disease at 36° C. than at 31° or 28° C.

In a later report (6) the writer showed that the mosaic disease of potatoes (rugose mosaic on Triumph and Green Mountain) is masked at constant temperatures above 24°–25° C., and that the optimal temperature for the disease is approximately 14°–18° C. Mention is also made in this paper of the relation of temperature to tomato mosaic, soy bean mosaic, pea-bean mosaic and clover mosaic.

Tompkins (9), working with the same chambers, studied particularly the relation of intermittent exposure of potato mosaic to high temperatures, and arrived at some particularly interesting results from such experiments. In general, it was found that masking and the tendency toward masking could be secured by comparatively short exposures to the higher temperatures of the chambers. This fact is not only significant from a practical point of view, but is of considerable scientific interest as well.

Recently, the writer has described the attenuation of tobacco mosaic (7) secured by exposure of inoculated plants to a constant temperature of 35° C. for ten days in the chambers.

The chambers have been extensively used for studies on the relation of temperature and humidity to the curing of tobacco, the details of which have not yet been published. In a minor way, they have also been used to a considerable extent for other problems, particularly by graduate students. Altogether, the experimental chambers have been very useful, and the results secured have well justified their construction, and have demonstrated the possibilities of further work along such lines either with similar or improved equipment.

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LITERATURE CITED

1. CROCKER, WM. Organization, equipment, dedication. Contributions from Boyce Thompson Institute for Plant Research, **1**: 9–58. 1925.
2. DICKSON, J. G. Making weather to order for the study of grain diseases. Wis. Agr. Exp. Sta. Bul. 379: 3–36. 1926.
3. HOTTES, C. F. A constant humidity case (Abst.). Phytopath. **11**: 51. 1921.
4. ———. Cooperative research in plant physiology and agronomy. Jour. Amer. Soc. Agron. **18**: 60–68. 1926.
5. JOHNSON, J. The relation of air temperature to certain plant diseases. Phytopath. **11**: 446–458. 1921.
6. ———. The relation of air temperature to the mosaic disease of potatoes and other plants. Phytopath. **12**: 438–440. 1922.
7. ———. The attenuation of plant viruses and the inactivating influence of oxygen. Science **64**: 210. 1926.
8. PELTIER, G. L., and R. W. GOSS. Control equipment for the study of the relation of environment to disease. Nebr. Agr. Exp. Sta. Res. Bul. 28: 3–16. 1924.
9. TOMPKINS, C. M. Influence of the environment on potato mosaic symptoms. Phytopath. **16**: 581–607. 1926.
10. TOTTINGHAM, W. E. Temperature effects in the metabolism of wheat. Plant Physiology **1**: 307–336. 1926.

PENICILLIUM INJURY TO CORN SEEDLINGS

HELEN JOHANN

When the seed-corn germination tests were made at the University of Wisconsin in the spring of 1927[1] and the records examined, attention was called to the possibility of *Penicillium* being a factor in the corn-disease problem in Wisconsin. On a number of ears *Penicillium* occurred with other fungi, while on 63 ears only the *Penicillium* was recorded. The average germination of the 63 ears infected with *Penicillium* was 79 per cent, and only 51 per cent of the kernels tested produced healthy seedlings. That is, 28 per cent of the seedlings from the kernels tested showed evident injury apparently from the *Penicillium*. Isolations from the diseased seedlings produced a high percentage of pure cultures of a green *Penicillium*.

The presence of *Penicillium* spp. within seed corn has been reported by Manns and Adams,[2] who recognized it as a parasite of the storage tissue of the scutellum and in some cases a limiting factor in germination. They, however, did not find it so consistently associated with seed corn that they considered it among the more important parasites.

Koehler[3] isolated a species of *Penicillium* from germinating corn kernels and as a result of his inoculation experiments he lists it among the fungi capable of producing scutellum rot. He says, "Scutellum rot of corn is a disease that occurs during the seedling stage of the plant and may be caused by a number of different organisms. On the germinator *Rhizopus* spp. are the most common causal agents." He finds that "Isolations from seedlings grown from susceptible seed in soil at 16° C. showed that species of *Mucor, Penicillium,* and *Fusarium* were the predominating organisms associated with scutellum rot."

Preliminary inoculation experiments in the Wisconsin soil-temperature tanks with Holbert's inbred strain B-1-1-3-R-10-1-12 and his cross No. 366,[4] a first generation hybrid of an inbred Leaming strain and strain A-1-1-2-R-1-1-9, indicate the probability that the *Penicillium* isolated in this laboratory may become a factor in the seedling blight problem under certain

[1] Germinator readings were made by Dr. J. G. Dickson and Mr. P. H. Senn.

[2] Manns, Thomas F., and J. F. Adams. Parasitic fungi internal of seed corn. Jour. Agr. Res. 23: 495–524. 1923.

[3] Koehler, Benjamin. Studies on the scutellum rot diseases of corn. Phytopath. 17: 449–471. 1927.

[4] Seed of Cross No. 366 was supplied by Dr. J. R. Holbert, Bloomington, Illinois. B-1-1-3-R-10-1-12 was grown at Madison, Wisconsin, by Mr. Paul Hoppe from seed received from Dr. Holbert in 1926.

TABLE 1.—*Reduction in germination and final stand as compared with the control in two lots of corn following seed inoculation with spore suspensions of Fusarium moniliforme, Trichoderma sp., and Penicillium sp.*

Inoculum	Seed[a]	16° C.		20° C.		24° C.		28° C.	
		Reduction in—		Reduction in—		Reduction in—		Reduction in—	
		germination	stand	germination	stand	germination	stand	germination	stand
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Fusarium moniliforme	x366	0	0	0	0	0	0	0	25
	B-1-1-3-R-10-1-12	0	6	7	20	13	12	[b]+6	0
Trichoderma sp.	x366	6	0	0	0	0	0	0	0
	B-1-1-3-R-10-1-12	[c]8	8	0	0	0	6	7	8
Penicillium sp.	x366	0	19	0	38	0	57	0	62
	B-1-1-3-R-10-1-12	[c]42	58	0	47	6	57	0	64

[a] 16 kernels planted in each group unless otherwise stated.

[b] + = increase.

[c] 12 kernels planted.

conditions. Germination was not seriously affected at any of the temperatures used. Reduction in the final stand occurred in all the tanks. At 28° C. and 24° C. the number of healthy plants remaining at the end of the experiment was less than 50 per cent in both lots of corn. The stand at 20° and 16° C., though not equalling that of the controls, was not reduced to so great an extent as was that at the higher temperatures.

In parallel inoculations in which *Fusarium moniliforme, Trichoderma* sp., and *Penicillium* sp. were used, the only consistent and severe injury occurred following the *Penicillium* inoculations (Table 1). This was of special interest in the case of Holbert's cross No. 366, as this vigorous cross had shown resistance to *Gibberella saubinetii, Diplodia zeae,* and *Pythium* sp. in a previous inoculation experiment.

The first symptom of invasion by the *Penicillium* was the light yellow-green color of the basal half of the upper leaves. This shaded into a normal green at the tip of the leaf. As the disease progressed, the tips and margins of the leaves became dry. Eventually all the leaves became dry without a striking change of color. Some of the seedlings reached the fourth-leaf or fifth-leaf stage before death occurred. A mass of *Penicillium* usually was plainly visible surrounding the tip of the kernel on the young seedlings which had been inoculated with a spore suspension at time of planting. Infection took place in the embryo region and proceeded up the mesocotyl. In some instances the mesocotyl was rotted at the base; in other cases it appeared to be clean. The fungus was isolated repeatedly from the embryo region and from portions of the mesocotyl which apparently were clean.

Sections, checked by platings of adjacent areas, showed the hyphae to be both inter- and intra-cellular. The mycelium was found to invade both the parenchyma and the vascular elements. Some sections indicated that the cells were killed in advance of the mycelium. This killing may possibly be due to oxalic acid formed by the fungus, as the organism produces numerous oxalate crystals within a short period of time when it is grown on potato dextrose agar.

A culture of the *Penicillium* was submitted to Dr. Charles Thom for determination. He replied that the culture resembled *P. oxalicum* Currie and Thom. According to these authors[5] *P. oxalicum* has been obtained from corn in Kansas and from moldy corn and cornmeal from Connecticut, Maryland, Virginia, and Illinois. They state that "it also occurs in soil cultures but evidently if it is a soil organism it is so well adapted to grow upon corn that it becomes a very common component of the flora of moldy corn and corn products."

[5] Currie, James N., and Charles Thom. An oxalic acid producing *Penicillium*. Jour. Biol. Chem. **22**: 287–293. 1915.

SUMMARY

1. *Penicillium* spp. have been reported on corn but have not generally been considered among the more important seed-corn parasites.

2. In seed-corn germination tests made at the University of Wisconsin in the spring of 1927, *Penicillium* frequently occurred and apparently injured the seedlings.

3. In inoculation experiments at different temperatures germination was not seriously affected. The greatest reduction of stand occurred at 28° C. and 24° C.

4. In parallel inoculations with *Fusarium moniliforme*, *Trichoderma* sp., and *Penicillium* sp., the only consistent injury occurred following inoculation with *Penicillium*.

5. Some seedlings reached the fourth- or fifth-leaf stage before death occurred. Infection took place in the embryo region and proceeded up the mesocotyl. The hyphae were both inter- and intra-cellular. Both the parenchyma and vascular elements were invaded. The cells were apparently killed in advance of the mycelium.

6. According to Dr. Charles Thom the *Penicillium* isolated from corn and used in the above experiments resembled *P. oxalicum* Currie and Thom.

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THE CROWN ELONGATION DISEASE OF THE PEONY

H. H. Whetzel

During late September, 1925, I received from a peony grower in the Middle West a recently dug root of a seedling peony plant about three years old. It was evidently diseased. The most striking symptom of the malady, and about the only one, was a marked elongation of the crowns with small weak buds at the tips. Again early in September, 1927, the same grower sent me another plant affected in the same way. On my request he sent me an additional half dozen or more plants showing the same symptoms. Several of these were of the variety Darkness, which showed the characteristic symptoms in a most striking manner (Fig. 1). A careful examination together with isolations from the roots failed to show the presence of any causal organism. Although I have examined many diseased peony roots in the course of the last 20 years I have never seen this particular disease from any other source so far as I recall. Whether it is contagious or not I can not say. The grower who sent me the specimen however, seems to think that the disease is on the increase in his plantings. He has observed that the disease seems to start on one side of a large clump and gradually involve the entire plant.

It seems desirable at this time to present a photograph and brief description of the symptomatology of this disease. Its most striking feature is a marked elongation of the crowns, the roots remaining apparently normal. These elongated crowns are much more numerous than the crowns on healthy plants, making a witches broom effect (Fig. 1). These numerous elongated crowns appear to originate by excessive bud formation about the base of the originally healthy crowns, which were rotted or partially destroyed in some way and later healed over.

The crowns not only elongate but branch more or less from adventitious buds along their sides. These secondary buds are very numerous. Feeding roots also develop from the elongated crowns as may be seen from figure 1, but they do not appear to be numerous and never thicken up like the storage roots in normal plants. The buds at the tips of these elongated crowns seem to be more advanced in development in the autumn than the buds on normal peony crowns.

In the spring, when the buds from these elongated crowns develop, they send up slender, weak shoots which seldom reach a height of more than 5 or 6 inches, with small dwarfed foliage and no flower buds. The roots apparently live for years but they never produce vigorous, blooming shoots.

A number of these diseased plants have been planted in my garden for further study and observation. Should this prove to be a contagious disease, its prompt eradication from plantations where it occurs is imperative.

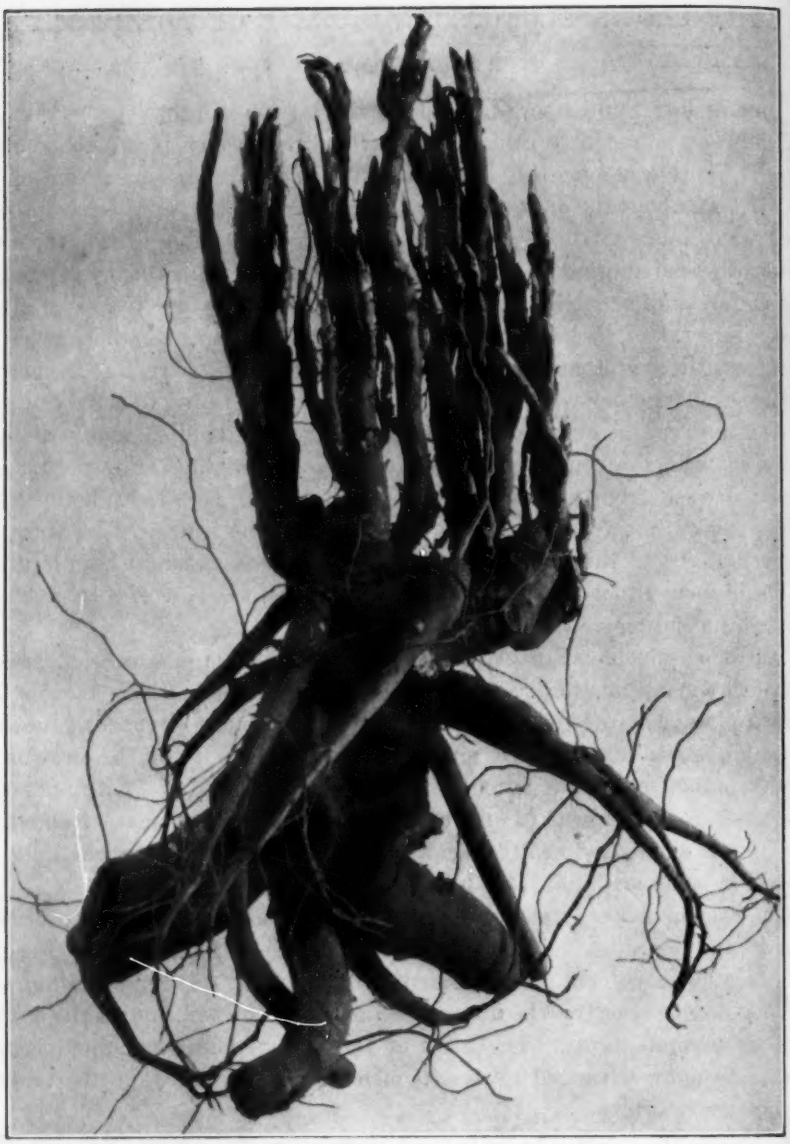

FIG. 1. Autumn condition of peony plant affected with crown elongation.

Affected plants never recover, and no attempts to propagate from them should be made. As they apparently never bloom, there would seem to be no reason for retaining them in a plantation.

Plant pathologists are urged to be on the look-out for this disease in peony plantations. The writer would be pleased to receive specimens of this disease from other sources.

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PHYTOPATHOLOGICAL NOTES

Killing of Strawberry Roots. Intermittently since 1922, observations and experiments have been made on root-killing of strawberry plants. These have been discontinued and the following summary of findings is presented.

All the varieties extensively grown in New York State are affected, and no consistent difference in amount of injury has been found.

The injury may appear in localized areas or on scattered plants throughout a planting. Typically the roots are killed from below upward and the plant tends to put out new roots higher up on the crown. In later stages the crown breaks down, beginning usually at the basal end from which the stolon has been detached. The entire plant frequently dies, particularly during a period of drouth at fruiting time or at times of heavy tax on the resources of the plant. Young plants sometimes die during the same season in which they are produced.

Attempts to isolate a pathogenic organism were made by G. R. Hoerner and by myself. Of 188 thrust cultures recorded from 12 sources, 68 plantings were sterile and 37 contained fungi of the Mucor type. Fungi of the genus *Cephalosporium* or closely related genera were obtained from at least five sources. In only one instance was *Rhizoctonia* isolated. Cultures from the interior of dying crowns were particularly likely to be sterile.

Nine different fungi and bacteria were used to make inoculations on plants grown in the greenhouse. None of these produced definite symptoms of disease.

Plants grown in pots with soil and with dead plants received from correspondents were as vigorous after several months as were the control plants.

Injured plants tended to recover when potted in greenhouse soil.

A planting of 18 varieties was made in the field at Ithaca, New York. Five of these were obtained from two plantations in which root-killing was abundant. The plants of one variety, Parson's Beauty, came from an area in which more than half the plants had died. Several fungi and soil and plant debris from four other sources were scattered over this planting. During three seasons never more than an occasional plant showed symptoms of root-killing. The variety, Parson's Beauty, was one of the most vigorous and fruitful of the lot. A few rods distant a second planting of the same varieties was made, to serve as a control for the first. Owing to poorer soil and cultural conditions the plants of the second planting were not so well grown or productive as were those of the first.

Strawberry plants are notably sensitive to soil and other environmental conditions. Factors which seem particularly to influence the occurrence of root-killing are: type and exposure of soil, low temperatures, application of lime, mulching practice.

It is concluded that root-killing of strawberries in New York State may result from a number of causes, frequently in combination, and that fungi in most cases at least play a minor rôle.—H. E. THOMAS, Department of Plant Pathology, Cornell University.

Verticillium wilt of heliotrope. In the fall of 1925 conspicuous dead areas were noticed in two large beds of heliotrope on the grounds of the Department of Agriculture in Washington. The plants in these areas were still upright, but the leaves and blossom clusters were black and wilted or shrivelled. Most of the stems were entirely brown, or brown in wide streaks. Examination showed a somewhat discolored vascular region and fungous mycelium in both cortex and wood.

From such stems a *Verticillium* resembling *V. alboatrum* was isolated and used to obtain successful infections on heliotrope in pots in the hothouse. The disease appeared again in 1926 and sporadically in 1927.

The fungus has a wide range of hosts but has not heretofore been reported on heliotrope. Van der Meer,[1] who gives a table of the distribution of Verticillium wilt among the dicotyledons, does not cite heliotrope nor any member of the Boraginaceae as a host.—MARY K. BRYAN, Assistant Pathologist, Laboratory of Plant Pathology, Bureau of Plant Industry, U. S. Dept. of Agriculture.

An improvement in the technique for feeding homopterous insects. Since the first report[2] was made on a method for feeding homopterous insects, which consisted of inclosing a solution in a membranous sack, it has been found that the technique can be advantageously adapted to petri dishes. The new method is to fill one-half of the petri dish with the solution and place a piece of the membrane over the dish, taking care that good contact is made with the surface of the liquid. A rubber band will hold the membrane to the dish. This can then be inclosed in any suitable type of cage, and for nymphs and wingless sucking insects it is in many ways an improvement over the sack which is hung free in the cage. It also permits the use of smaller quantities of solution—as low as two or three cc. if a small petri dish is used.

[1] Van der Meer, J. H. H. Verticillium wilt of herbaceous and woody plants. Meded. Landbouwhoogeschool, Wageningen 282. 1925.

[2] Carter, W. A technic for use with homopterous vectors of plant disease, with special reference to the sugar-beet leaf hopper, *Eutettix tenellus* (Baker). Jour. Agr. Res. 34: 449–453. 1927.

The fishskins described in the original article are expensive and somewhat difficult to obtain, and for that reason a continued search has been made for a suitable substitute. Such a substitute, for most purposes, was found in Baudruche Capping Skins.[3] This product comes in sheets averaging 8 or 9 inches in width and from 30 to 36 inches or more in length. Sacks can easily be made from these flat sheets, and for the petri-dish method they are readily cut to fit a petri-dish of any size.

One difficulty has been encountered with this skin. When there is considerable pressure from a quantity of solution inclosed in a sack of this material there tends to be more oozing from the feeding punctures than there would be with the use of the fishskins. There is not this disadvantage, however, in the petri-dish method, as the material is not subjected to any pressure from a large volume of solution. This material is very similar in appearance and texture to the fishskins but perhaps not so strong, and should it prove generally satisfactory it will be very much cheaper and more easily obtained than the original material.—WALTER CARTER, Bureau of Entomology, U. S. Department of Agriculture, Twin Falls, Idaho.

Phytophthora, Pythium, and Pythiacystis species as stock cultures. Supervision of cultures for such an undertaking as the American Type Culture Collection necessitates consideration of artificial culture media maturity of the organism at the time the culture is placed in storage, humidity conditions in the storage compartment, when possible, and storage tem peratures. Observations of stock fungous cultures to be even of suggestive value must be made with a background of continuous experience which is generally not attainable.

It seems possible at present to make note of the reactions of some species of *Phytophthora* and *Pythium* to storage temperatures. This note is based on one series of transfers of stock cultures of these two genera. The data included here were discussed with Dr. Charles Drechsler of the United States Department of Agriculture.

The species on hand were transferred between July 26 and 28 to potato agar. The medium was prepared by adding 500 gms. of pared and finely sliced potato to 1,000 cc. of water, the whole being heated with steam for 45 minutes and then filtered through two or three layers of wet cheesecloth. After filtration 15 gms. of agar was added, and the medium sterilized at 15 lbs. for 15 minutes. Transfers were made by cutting out a piece of the old culture somewhat more than 5 mm. square and placing it at the base of the fresh slant where a small pool of liquid had accumulated. Three sets of *Phytophthora arecae* Pethybridge, *P. cactorum* Schröter (strains 1,

[3] The samples used were transparent Baudruche marked 1-A and 1-B and are manufactured by Paul Troeder, Belleville, New Jersey.

2, and 3 of Leonian[4]), *P. capsici* Leonian, *P. colocasia* Raciborski, *P. erythroseptica* Pethyb., *P. faberi* Maub., *P. fagi* Hartig, *P. infestans* DeBary, *P. palmivora* Butler, *P. parasitica* Dastur, *P. parasitica* var. *rhei* Godfrey (strains I through V of Leonian[4]), *P. pini* Leonian, *Pythium debaryanum* Hesse var. *pelargonii* Brauns, *Pythiacystis citrophthora* Sm. and Sm. were transferred in this manner and allowed to develop at room temperature (26 to 32° C.) from two to three weeks. One set was then placed at 0° C., another at 7° C., and the third retained at the room temperature (26 to 32° C.). On retransferring at the end of the two and a half months, viability among the cultures was as follows: of those stored at room temperature, only *Pythium infestans* was no longer viable; of the transfers stored at 7° C., *Phytophthora arecae, P colocasiae, P. parasitica-rhei* V, and *P. infestans* did not grow on retransfer; of those stored at 0° C., *Phytophthora capsici, P. parasitica-rhei* III and *P. pini,* and the species which failed to develop at 7° C., did not grow.

All these cultures or strains were obtained from Leonian by the Collection in 1925, and are discussed in his paper[4] of that year. Accordingly, they have been maintained in culture tubes for over two years or more by the Collection and others. Recent determination of their ability to produce sound fruiting structures in cornmeal agar does not correlate with their ability to remain viable at the three temperatures under discussion. Accordingly, we may conclude that 0 and 7° C. are unsatisfactory temperatures for the maintenance of these species of *Pythium* and *Phytophthora* under the conditions to which they were submitted and probably for all conditions of artificial maintenance.—MARGARET B. CHURCH, Bureau of Chemistry and Soils, U. S. Department of Agriculture, and MARIO SCANDIFFIO, of the American Type Culture Collection.

[4] Leonian, Leon H. Physiological studies on the genus *Phytophthora.* Amer. Jour. Bot. **12**: 444–498. 1925.